Preface

In recent years, carbohydrates in the diet have been the focus of much research from a nutritional point of view, much more so than ever before. Both beneficial and harmful properties have been attributed to carbohydrates, and diets low in carbohydrates are advocated by some as healthier. Carbohydrates in food can include anything from the simple monosaccharide glucose to the very complex polysaccharides found in cell walls; therefore, their roles in food products, both nutritionally and functionally, cannot be viewed as simply “good” or “bad.” The same properties cannot be attributed to all carbohydrates, and all carbohydrates, therefore, cannot be regarded as a single component. Anyone working with product development in the food industry usually has to deal with several different carbohydrates in the same product, and it is seldom possible to choose one simple or even well characterized carbohydrate. This book deals with all these carbohydrates: monosaccharides and disaccharides, cell-wall polysaccharides, polysaccharides described as gums and hydrocolloids, and starch.

An awareness of the health aspects of our diet has grown since the first edition of this book. A huge challenge today is to combine health benefits with sensory properties that appeal to the consumer; moreover, the food product should also be convenient for the consumer. Those who work with carbohydrates in food must have knowledge about the chemical analysis of carbohydrates, as well as their physicochemical properties and how carbohydrates can be used in product development for the benefit of the public. It is my hope that this book will inspire product developers, nutritionists, and food scientists to achieve further success in this very important field.

Ann-Charlotte Eliasson
The Editor

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1.1 INTRODUCTION

The determination of mono- and disaccharides is one of the most frequently required analyses in the food analysis laboratory and has considerable application in nutritional and biochemical studies. The variety of food and beverage products from food manufacturers continues to expand. This variety, combined
with raised expectations of quality and consistency from the consumer, has created a need for analytical methods that provide specific data on the composition of both raw materials and final products. In the latter case, such analyses may also be driven by legislation regarding nutrition labeling information, reinforced by increasing research activity into the dietary and nutritional significance of carbohydrates in general.

Analyses for mono- and disaccharides must be applicable to simple ingredients, complex processed foods, and fractions or components isolated in nutritional studies. In addition, the monitoring of manufacturing processes may make further demands with respect to rapid analysis; thus, determination of mono- and disaccharides in foods and related matrices requires specific, quantitative, and rapid analytical methodology.

This chapter provides a brief outline of chemical and biochemical methods but with a strong emphasis on chromatographic methods. The comparative and complementary uses of gas and liquid chromatographies are discussed, and the methods and techniques employed are described.

1.2 ANALYTICAL OBJECTIVES

The analytical information most commonly sought with regard to the mono- and disaccharide composition of foods requires determination of some or all of the following: glucose, fructose, sucrose, lactose, and maltose. Analysis for these sugars must be applicable to a wide range of foods, including cereal products, dairy products, fruits, vegetables, preserves and confectionery, and beverages. The methods deployed should also encompass the determination of less common sugars (e.g., pentoses) and sugar alcohols (e.g., sorbitol). In addition to raw data on composition, interpretation of the data allows, for example, deducing the extent of inversion or use of invert sugar from fructose, glucose, and sucrose concentrations and gives an indication of nonfat milk powder content from the lactose concentration.

Monosaccharide analysis is often employed for the final determination of carbohydrate polymer constituents, thus enabling specific groups or types of carbohydrates to be measured. Dietary fiber determination (as nonstarch polysaccharide) is perhaps the most common application in current practice, but others include the measurement of pentosans in cereal products and gums and thickening agents in composite foods.

Analytical methods applicable to routine determinations or quality control should be differentiated from research or investigative methods. In the former sample, throughput is often important so methods should be readily automated. The latter type may involve complex mixtures requiring rigorous qualitative investigation or the detection and quantification of very low concentrations of specific and perhaps less common saccharides.
Although chromatographic methods may currently be regarded as the methods of choice, physical, chemical, and biochemical methods still play a role in the analysis of carbohydrates. Indeed, the Association of Official Analytical Chemists International [1] refers to a wide range of methods applicable to a variety of foods. Physical methods, primarily polarimetry and refractometry, are of limited use for mixtures of mono- and disaccharides, as they obviously lack specificity; however, chemical and biological methods can provide specific analyses by either direct or indirect measurement. These are briefly reviewed below followed by detailed sections on chromatographic methods.

1.3 CHEMICAL AND BIOCHEMICAL METHODS OF ANALYSIS

1.3.1 CHEMICAL METHODS

Reducing sugar determination, although not saccharide specific, is nevertheless often carried out on foods. This analysis most commonly indicates glucose and fructose concentrations or, in milk products, lactose and galactose concentrations. Given their propensity for reaction with amino groups in the Maillard reaction and the resulting undesirable development of brown colors and off flavors, determination of reducing sugars provides a measure of this potential problem.

The most common procedure is the Lane and Eynon titration method, which is based on the ability of reducing sugars to react with an alkaline cupric complex and reduce it to cuprous oxide. This is frequently carried out using Fehlings 1 solution (copper sulfate) and Fehlings 2 solution (sodium hydroxide and sodium potassium tartrate). Time, temperature, and reactant concentrations must be controlled (details can be found in the International Commission for Uniform Methods of Sugar Analysis 1978 [2] method).

Sucrose can also be determined using Lane and Eynon titration. Two determinations of reducing sugars, before and after inversion, are necessary. The sucrose content is the difference in reducing sugars, corrected by 0.95, the hydrolysis factor.

1.3.2 BIOCHEMICAL (ENZYMATIC) METHODS

Individual sugars can be determined by methods based on a specific property of the saccharide, and the use of enzymes provides such specificity. These methods involve treatment of the sugar with the appropriate enzyme and then determination of the reaction products by, for example, spectrophotometry. Examples of this approach are outlined below.
1.3.2.1 Glucose

Glucose (specifically β-D-glucose) can be oxidized by glucose oxidase to produce hydrogen peroxide. This is then reacted with a dye in the presence of peroxidase, and the concentration of the colored product (measured spectrophotometrically) is proportional to the initial glucose concentration. As an alternative, glucose is converted by hexokinase to glucose 6-phosphate, which is then oxidized by nicotinamide adenine dinucleotide phosphate (NADP) in the presence of glucose-6-phosphate dehydrogenase, and NADP is reduced to NADPH [3]. The concentration of the latter is measured spectrophotometrically at 340 nm.

1.3.2.2 Fructose

Fructose can be measured by employing the second method outlined above after the initial formation of fructose-6-phosphate. This is then converted to glucose-6-phosphate with phosphoglucose isomerase, and the sequence continues as for glucose.

1.3.2.3 Lactose

Determination of lactose is based on its hydrolysis by β-galactosidase to galactose and glucose. Galactose is then measured by its reduction of NADP in the presence of β-galactose dehydrogenase [3].

1.3.2.4 Maltose

The glucose procedure can be used for the determination of maltose after an initial hydrolysis to glucose with maltase (α-glucosidase) [3]. Sucrose, if present, must be determined separately, because with maltase treatment it is also a source of glucose.

1.3.2.5 Sucrose

Invertase (β-fructosidase) treatment of sucrose produces glucose and fructose, and the glucose can be measured by the hexokinase methods [3].

1.4 CHROMATOGRAPHIC METHODS

Methods for determination of carbohydrates have employed all of the chromatographic techniques; however, the instrumental methods of gas–liquid chromatography (GLC) and high-performance liquid chromatography
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(HPLC) have largely replaced the earlier methods of paper chromatography and thin-layer chromatography (TLC) for the determination of mono- and disaccharides due to their advantages with regard to separation efficiencies, quantification, and speed of analysis. Where very many samples must be analyzed and only qualitative or semiquantitative data are required, TLC may be the most efficient procedure. Indeed, the use of this technique was demonstrated by Jadhav et al. [4], who reported the determination of glucose, fructose, and sucrose in sugar cane juice by high-performance TLC.

Two reports in particular may be considered to have been the catalysts for development of GLC and HPLC methods. In 1963, Sweeley et al. [5] described the application of GLC to a wide range of saccharides, and this report was then followed by many papers describing diverse applications of GLC. In 1976, Schwarzenbach [6] reported the HPLC of low-molecular-weight saccharides using an amino-bonded silica column. Since then, similar columns and alternatives have become widely available, and many reports have appeared describing the determination of mono- and disaccharides. Over the past 15 years, HPLC has clearly replaced GLC as the most commonly applied technique. Indeed, continued progress in HPLC techniques (driven primarily by major improvements in detectors) may suggest that GLC methods are becoming obsolete, but in practice this is not the case. Although HPLC has become the method of choice for many determinations, GLC procedures continue to be used in carbohydrate analysis.

The two techniques offer different selectivities with respect to resolution and can thus be considered complementary. Where very high resolution is required, capillary column GLC offers considerable scope. The most frequently employed GLC application in current use is the determination of monosaccharides of nonstarch polysaccharide as reported by Englyst et al. [9] and Theander et al. [10], who employed capillary GLC for the final determination.

Where confirmation of identity is required, capillary GLC in conjunction with mass spectrometry (GLC-MS) likewise provides a practical solution, as it is much more widely available than HPLC–mass spectrometry (LC-MS). When comparing GLC and HPLC methods for determination of mono- and disaccharides, several aspects must be considered, including sample preparation, speed of analysis, sensitivity, and specificity (influence of coextractives). Overall, practical considerations favor HPLC, at least for most routine analyses, with GLC being employed in specialized areas where its advantages are beneficial.

Regardless of the absolute merits of GLC and HPLC, both techniques are used in many laboratories for mono- and disaccharide determinations. The following sections review the application of GLC and HPLC to the analysis of mono- and disaccharides and describe practical details of the analytical methods.

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1.5 GAS CHROMATOGRAPHIC DETERMINATION OF MONO- AND DISACCHARIDES

1.5.1 DEVELOPMENT OF TECHNIQUES AND PROCEDURES

This review of developments in methodology is not exhaustive but highlights those of greatest significance. Detailed consideration of practical aspects can be found in Section 1.5.2 and Section 1.5.3. Since the report by Sweeley et al. [5], a vast number of papers have been published on the application of GLC to the analysis of mono- and disaccharides. Reports of GLC methods now appear much less frequently than those for HPLC methods, but some examples include the determination of sugars in persimmons by Senter et al. [7] in 1991 and a comprehensive paper on disaccharides by Garcia-Raso et al. [8] in 1992.

Despite the continuing progress in GLC, particularly with respect to capillary column technology, no truly major advances in the basic methodology for mono- and disaccharide analysis by GLC have been made for more than 10 years. The development of methods for mono- and disaccharides analysis by GLC has encompassed the following aspects:

1. Reactions for preparation of volatile derivatives
2. Reactions for initial conversion of sugars to improve the subsequent qualitative or quantitative determination
3. Quantification with regard to the use of internal standards

An essential prerequisite for analysis by GLC is the preparation of volatile derivatives. The literature clearly reveals the predominance of the O-trimethylsilyl (O-TMS) ether derivatives and the common use of the O-acetyl derivatives. Other derivatives, including O-trifluoroacetyl, have been rarely used.

The initial paper by Sweeley et al. [5] described a simple method for O-TMS preparation using a mixture of hexamethyldisilazane (HMDS) and trimethylchlorosilane (TMCS). An alternative procedure was developed by Brobst and Lott [11] that employs HMDS with trifluoroacetic acid (TFA), which tolerates the presence of water, as the catalyst. Alternative procedures including the use of N,O-bis-trimethylsilyl-trifluoroacetamide (BSTFA) and N-trimethylsilylimidazole (TMSIM) have also been reported.

Reducing sugars exist as a mixture of two or more isomers in solution, giving rise to multiple peaks and a complex chromatogram for mixtures of saccharides. In those cases where it is advantageous to simplify the chromatogram and hence quantification, preliminary modification of the sugars, ideally to result in a single peak for each saccharide, is required. Two approaches have been favored: the reduction to alditoles, as first reported by Sawardeker et al. [12], and the formation of oximes, extensively studied by Zurcher et al. [13]. The formation of acetate derivatives of saccharides and their use in GLC...
analysis have been established. In most cases, this process has been in conjunction with their prior reduction to alditols, as used in the final determination of monosaccharides of nonstarch polysaccharides.

An important advance has been the widespread adoption of fused silica capillary columns for GLC. This has greatly reduced the problems associated with the analysis of complex mixtures. Adams and Jennings [14] hinted at this development as early as 1975, with their use of glass capillary columns for the analysis of mixtures of disaccharides.

1.5.2 Sample Treatment and Derivative Formation

1.5.2.1 Sample Treatment

Although it might be inferred that sample treatment for both GLC and HPLC determination would be similar, the differences are sufficient to merit separate descriptions in this chapter. The sample treatment required prior to GLC determination is very dependent on the type of sample and its water content. Samples of high sugar content, such as confectionery, preserves, and syrups, can be silylated without any initial treatment, particularly when the Brobst and Lott [11] procedure is employed. With the greater sensitivity attainable with capillary column methods, this approach can be extended to samples of lower sugar content such as fruit liquors (approximately 10% solids). The acetylation of saccharides using acetic anhydride and pyridine requires anhydrous conditions, but the later method of Bittner et al. [15], in which the catalyst is N-methylimidazole, offers a rapid procedure in aqueous solution.

In general, aqueous samples require at least partial removal of water prior to oximation or silylation. Mild conditions, such as low-temperature vacuum drying, must be maintained to prevent changes or losses in the sugars.

Direct or in situ reaction with samples can often be used for TMS derivatization, but precautions with such applications are necessary. Constituents other than saccharides may be extracted and derivatized, hence causing erroneous results to be obtained. For example, fruit acids may be converted to TMS esters having chromatographic behavior similar to that of monosaccharides. In addition, the small sample amounts processed with direct or in situ methods may not be representative, particularly for composite foods.

For many foods, the direct approach above is inappropriate, and sample treatment with the optional use of steps 1, 3, and 4 below is required:

1. Extract with hexane to remove fat.
2. Extract with water or 80% ethanol (ideally with incorporation of the internal standard).
3. Remove interfering acids using lead acetate.
4. Remove proteins using Carrez reagent or equivalent.
5. Make extract to standard volume, lyophilize aliquot, and derivatize.

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1.5.2.2 Derivative Formation

Section 1.5.1 above briefly reviewed the range of possible derivatives of mono- and disaccharides; the following section provides a more detailed consideration of their selection and preparation. The two essential prerequisites for GLC determination of mono- and disaccharides are the quantitative preparation of volatile derivatives and the chromatographic resolution of the resulting mixture. This is often more complex than predicted due to the anomer and ring isomer forms of saccharides and the multiple peaks produced upon GLC analysis. Complete resolution is now more likely to be realized with capillary columns, but quantification based on two or more peaks per component may incur greater error than for a single peak. Simplification of the GLC of mono- and disaccharides by an initial conversion step can therefore be an aid to both qualitative and quantitative analysis. It appears that recent practice has rationalized the range of derivatives employed to:

- **Initial derivative:**
  1. Alditol by reduction
  2. Oxime (or methoxime)
- **Volatile derivative:**
  1. O-Acetyl
  2. O-Trimethylsilyl

Reagents and reaction conditions have been devised to produce rapid quantitative yields of both initial and volatile derivatives. For example, the Englyst et al. [9] procedure for determination of the monosaccharides of nonstarch polysaccharide demonstrates ideal criteria for GLC determination, as shown by the monosaccharide standards in Figure 1.1 analyzed as alditol acetates. If an initial derivative is to be employed, it must obviously improve the analytical performance. Thus, reduction is inappropriate for mixtures in which both fructose and glucose are present, as the reduction products are mannitol and sorbitol from the former and sorbitol from the latter.

Oxime formation, as reported by Zurcher et al. [13], can be a very useful aid in the analysis of mono- and disaccharides. It has the practical benefit that reaction in pyridine allows the oxime- and O-TMS derivatives to be prepared in simple consecutive steps. Whereas early reports indicated that GLC (packed columns) of oxime-TMS derivatives gave only one peak for most saccharides, Adams and Jennings [14] found that capillary columns resolved methoximes into major and minor peaks; nevertheless, the analysis may still be simplified by this modification. The determination of the common food sugars (fructose, glucose, sucrose, maltose, lactose) can be assisted by use of the oximes, as can be seen by comparing Figure 1.2 and Figure 1.3. The measurement of fructose is simplified, and the resolution of sucrose from lactose is greatly enhanced. This is particularly advantageous when lactose is determined in an excess of sucrose.
The use of oximes has limitations, however, and the analyst must be aware of these in selecting the analytical method. The presence of mannose and galactose makes determination more difficult, as indicated in Figure 1.2 and Figure 1.3. A further potential difficulty, illustrated by Figure 1.4 and Figure 1.5, is the analysis of sugars in apricot in which sorbitol is also present. The retention time of fructose as the oxime is now very similar to that of sorbitol. If fructose is present in great excess, a loss of resolution may prevent the determination of sorbitol. If sorbitol is not present (as in the case of currants and berry fruits), oxime formation may be beneficial. The advantages and disadvantages of oxime formation are further illustrated by the analysis of yogurt, as shown in Figure 1.6 and Figure 1.7. Oxime formation aids the determination of mono- and disaccharides (particularly for yogurts with added sucrose), except that the minor oxime components of galactose and glucose now coelute, whereas their anomers are fully resolved as O-TMS ethers. It should be recognized that alternative stationary phases will influence the merits and demerits discussed above. As an alternative, it may be concluded that, where mannose and galactose are present, the preferred analytical
route is by reduction to alditols. It is the authors’ view that, with the enhanced resolution of capillary columns, analysts should endeavor to achieve the required analytical performance by optimizing the conditions for GLC determination,

**FIGURE 1.2** Separation of O-TMS derivatives of mono- and disaccharides. Column, 25 m × 0.2 mm × 0.25 µm; film, Ultra-2 (5% phenyl methylsilicone); temperature, 150°C for 1 min, ramp at 4°C/min to 300°C. 1–4, Arabinose; 5, 6, xylose; 7, mannose + fructose; 8, 9, fructose; 10, 11, galactose; 12, glucose; 13, galactose + mannose; 14, mannitol; 15, sorbitol; 16, glucose; 17, phenyl-β-D-glucopyranoside; 18, sucrose + lactose; 19, 20, maltose; 21, lactose.

**FIGURE 1.3** Separation of oxime-TMS derivatives of mono- and disaccharides. Conditions as for Figure 1.2. 1, Xylose; 2, xylose + arabinose; 3, arabinose; 4, 5, fructose; 6, mannose; 7, galactose; 8, glucose; 9, mannose + galactose + glucose; 10, phenyl-β-D-glucopyranoside; 11, sucrose; 12, 13, lactose; 14, 15, maltose.
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including optimum selection of the stationary phase, in preference to the use of oximes or other initial derivatives. This approach will allow for simpler preparation and more rapid overall determination.

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**FIGURE 1.4** Separation of O-TMS derivatives of monosaccharides of apricot puree. Conditions as for Figure 1.2. 1, Fructose (major component); 2, unknown; 3, glucose; 4, sorbitol; 5, glucose.

**FIGURE 1.5** Separation of oxime-TMS derivatives of monosaccharides of apricot puree. Conditions as for Figure 1.2. 1, Unknown; 2, sorbitol; 3, 4, fructose; 5, 6, glucose.
Literature reports have consistently shown a predominant use of TMS derivatives due to their ease of preparation, stability, and chromatographic properties. The properties of acetates are also highly favorable for GLC analysis, and they are now more easily and rapidly prepared by the procedure of Bittner et al. [15] using N-methylimidazole as catalyst, compared to the earlier procedures using pyridine. Acetate derivatives are particularly favored for the resolution of complex saccharide mixtures when used in conjunction with

FIGURE 1.6 Separation of O-TMS derivatives of mono- and disaccharides of fruit yogurt. Conditions as for Figure 1.2. 1–3, Fructose; 4–7, galactose; 6, 8, glucose; 9, 10, lactose.

FIGURE 1.7 Separation of oxime-TMS derivatives of mono- and disaccharides of fruit yogurt (no added sucrose). Conditions as for Figure 1.2. 1, 2, Fructose; 3, galactose; 4, glucose; 5, galactose + glucose; 6, 7, lactose.
reduction to alditols. The reagent systems most commonly employed for preparation of O-TMS and O-acetyl derivatives of saccharides are given below:

- **O-Acetyl**
  - Acetic anhydride and pyridine (3:1 or 1:1)
  - Acetic anhydride and N-methylimidazole (10:1)

- **O-Trimethylsilyl**
  - HMDS and TMCS (2:1)
  - HMDS and TFA (9:1)
  - BSTFA
  - N-Methyl-trimethylsilyl-trifluoroacetamide (MSTFA)
  - N-Methyl-N-trimethylsilyl-heptafluorobutyramide (MSHFBA)
  - TMSIM

Trimethylchlorosilane can be used as a catalyst with reagents 3 to 6, but its use is essential with HMDS.

The amide reagents may give multiple peaks for aldose and ketose sugars and are not recommended, but they can be used following reduction or oxime formation. Zurcher et al. [13] prepared the oximes and then used MSHFBA and TMCS (4:1) to make the TMS ethers. These reagent systems can all be used alone or in a solvent, commonly pyridine. Formation of TMS ethers is usually complete in 5 to 30 min, depending on the nature of the sample, and can be assisted by heating at 60 to 70°C. Preparation of TMS ethers of mono- and disaccharides is thus simply and rapidly carried out and applicable to the analysis of many samples by GLC.

### 1.5.3 Qualitative and Quantitative Analysis

#### 1.5.3.1 Gas Chromatography and Qualitative Analysis

The chromatographic requirements for determination of mono- and disaccharides in foods and related materials range from the simple, such as for corn syrup, to the complex, such as for composite foods containing several sugars. The use of packed columns for analysis entails evaluation of many stationary phases to resolve complex mixtures; however, the use of capillary columns, with their greatly increased resolution, has enabled the use of nonpolar to medium-polarity columns for O-TMS derivatives and the use of high-polarity columns for O-acetyl derivatives. The advances in GLC capillary columns and development of HPLC methods for sugar analysis have occurred over a similar time period; reported applications of capillary column GLC analysis to carbohydrate analysis are limited, and no comprehensive review has appeared. Nevertheless, the obvious advantages of resolution, speed of analysis, and enhanced sensitivity suggest that, where GLC procedures are used, capillary columns should now be the norm. Reports of capillary column use
prior to the era of fused silica columns provide an indication of the most suitable stationary phases. Tesarik [16] in 1972 reported the resolution of pentoses and hexoses as TMS ethers using columns of Se-30, OV-101, and XE-60. Reduced monosaccharides as acetates and TMS ethers and reduced disaccharides as TMS ethers were analyzed by Adam [17] using OV-101. Published capillary column separations indicate that mixtures of TMS ethers can be resolved using columns of methylsilicones or phenylmethylsilicones (e.g., OV-101, OV-17), and sugar acetates can be resolved using the polar phase SP-2330 or an equivalent. The chromatograms shown in Figure 1.2 to Figure 1.7 were all obtained using a 5% phenyl-substituted methylsilicone column with some apparent deficiencies in resolution. These may be overcome by the use of more polar phases such as 50% phenyl-substituted methylsilicone (e.g., OV-17).

1.5.3.2 Detection and Quantification

1.5.3.2.1 Detection
For the great majority of mono- and disaccharide analyses performed using GLC, the flame ionization detector (FID) has been used. This allows for the routine determination of sample components down to the 100-ppm level using capillary columns which is more than adequate sensitivity for most applications. Further gains in detection limit may be achieved by exploiting the benefits of splitless or on-column injection techniques with capillary columns.

Highly specialized areas of carbohydrate research may demand greater sensitivity or selectivity than can be attained by use of the FID. The nitrogen-selective thermionic detector, for example, can be applied to the analysis of oxime-TMS derivatives to enhance both sensitivity and selectivity in comparison to FID analysis. Morita and Montgomery [18] reported the application of this detector to the determination of peat monosaccharides and claimed an up to 30-fold reduction in detection limit compared to FID analysis. An alternative approach was reported by Eklund et al. [19], who employed the electron capture detector for the analysis of the trifluoroacetates of monosaccharides and achieved subpicogram detection. These applications, particularly the latter, are obviously very extreme examples of monosaccharide analysis. The major advances in HPLC detectors in the past 10 years, with respect to both sensitivity and selectivity, have obviated many of the analytical criteria that previously favored the use of GLC determination.

1.5.3.2.2 Quantification
Quantification of mono- and disaccharides determined by GLC methods is preferably performed by reference to an internal standard. The ideal internal standard should be chemically similar to the analytes, such that it is also derivatized and must be completely resolved from all sample components. A further criterion is its absence from all samples to be analyzed. Internal
standards reported in the literature have included uncommon and substituted monosaccharides and other polyhydroxy compounds.

For the determination of pentoses and hexoses as their alditol acetates, Englyst et al. [9] have used allose as the internal standard, and Theander et al. [10] favored inositol. Various internal standards have been employed for the determination of mono- and disaccharides as their TMS ethers, but the most frequently reported internal standard is clearly phenyl-β-D-glucopyranoside.

These internal standards are ideally blended with samples in water or pyridine (if appropriate), such that all subsequent sample treatment steps are common to the analytes and standard, thus minimizing possible sources of error in quantification.

1.5.4 APPLICATIONS AND PRACTICAL PROCEDURES FOR GLC ANALYSIS

1.5.4.1 Applications

Following the pioneering work of Sweeley et al. [5] and others, a great many reports were published on the application of GLC to the determination of mono- and disaccharides; however, these have declined in number due to the lack of further novel applications and the continued progress and application of HPLC methods. Reported applications have embraced a wide range of analyses covering many food and food-related matrices. These range from the determination of a single common sugar to the determination of several sugars and sugar alcohols in composite foods. Methods have also been devised for the determination of the monosaccharide composition of carbohydrate polymers. This very brief review of applications provides typical examples of the GLC methods that have been reported over a span of 30 years. Their current usefulness depends on the analyte/matrix combination and the chromatography equipment available in the food analysis laboratory.

Analysis of glucose syrups is now carried out in nearly all laboratories by HPLC, but determination of the anomer ratio of α-glucose to β-glucose, which may influence physical properties, is more readily carried out by GLC. A method for glucose determination using sorbitol as the internal standard was described by Alexander and Garbutt [20]. When carried out using premixed silylation reagent (pyridine/HMDS/TMCS, 10:2:1) to give rapid derivatization, the initial anomer ratio is maintained and quantitative determination of each anomer is obtained.

The analysis of a range of foods (tomatoes, potatoes, apples, cabbages, and carrots) for fructose, glucose, and sucrose using TMS ethers was reported by Davison and Young [21]. Demaimay and Lebouteiller [22] employed the TMS ethers of oximes for the analysis of milk, must, apple juice, jam, and chocolate candy. The application of GLC to the analysis of minor sugars was
demonstrated by Doner et al. [23], who measured the maltose/isomaltose ratios in honeys as a means of detecting adulteration with high-fructose corn syrup. A further report regarding employing oximes as their TMS derivatives was published by Wong Sak Hoi [24], who determined fructose, glucose, and sucrose in cane sugar products. The acetates were the preferred derivative for the determination of sorbitol, mannitol, and xylitol in chewing gum, as reported by Daniels et al. [25]. A wide range of yogurts was analyzed by Li et al. [26], who employed oximes and TMS derivatives with phenyl-β-D-glucopyranoside as the internal standard. The oxime-TMS derivatives were employed by Chapman and Horvat [27] for the determination of a wide range of sugars in apples, pears, peaches, and sweet potatoes. Identifications were confirmed by GLC–mass spectrometry. A similar approach to the analysis of persimmons was described by Senter et al. [7], who determined arabinose, galactose, glucose, fructose, and sucrose against phenyl-β-D-glucopyranoside as the internal standard. A highly specialized application was reported by Glassgen et al. [28] in which the monosaccharide components of anthocyanins and flavonols were determined as their alditol acetates.

1.5.4.2 Methods

Although many methods have been reported in the literature, most of the applications of GLC to the analysis of mono- and disaccharides in foods fall into one of the following categories:

- Determination as TMS ethers with direct derivation
- Determination as TMS ethers following the preliminary formation of the oximes of reducing sugars
- Determination as acetates following the initial reduction of reducing sugars to alditols

Outlined procedures for the most commonly used methods are given below. Alternative procedures may often be employed but those that follow have been used successfully in the authors’ laboratory.

1.5.4.2.1 Determination of Mono- and Disaccharides as TMS Ethers

Sample preparation and derivatization:

1. Direct derivation is possible for many foods. Extract and clean up the sample as necessary (see Section 1.5.2.1), depending on the nature of the sample and its sugar content.
2. Take a sample aliquot ideally equivalent to at least 5 mg saccharides for capillary column analysis (or 50 mg for packed columns) but containing not more than 40 mg water.
3. Dissolve the sample in pyridine (1.0 ml) containing phenyl-β-D-glucopyranoside (1 to 5 mg/ml) as the internal standard.
4. Add HMDS (0.9 ml) and TFA (0.1 ml), mix vigorously, leave to react for 30 min, and proceed with GLC determination.

**Gas chromatography operating conditions:**
1. Column — 25 m × 0.25 mm × 0.25 μm film, OV-1 or OV-17 (methylsilicone or phenylmethylsilicone) liquid phase
2. Carrier gas — Hydrogen or helium at 10 psi (gas velocity of 40 cm/s or 25 cm/s, respectively)
3. Injection — Split-mode operation, split ratio 50:1, 0.5- to 1.0-μl injection at 250°C
4. Detector — Flame ionization detector at 300°C
5. Oven temperature — 150°C for 1 min, ramp to 300°C at 4°C/min

**Calibration and quantification:**
1. Prepare calibration standards by taking aliquots of aqueous solutions of reference saccharides equivalent to 1 to 5 mg, and dry if necessary. (These can be blended in a single calibration mixture if the chromatography and separation have been previously established.) Proceed as in steps 3 and 4 in sample preparation and derivatization above.
2. Determine response factors relative to the internal standard by analysis as for the gas chromatography operating conditions, above.
3. Quantify the amounts of each mono- and disaccharide in the sample from the peak area data and response factors.

**1.5.4.2.2 Determination of Mono- and Disaccharides as Oxime-TMS Ethers**

This method is very similar in procedure to that described for TMS ethers above, except for the following steps (direct derivatization of water-containing samples is not applicable):

1. Take a sample aliquot ideally equivalent to at least 5 mg saccharides for capillary column analysis (or 50 mg for packed columns), and remove water by vacuum drying.
2. Dissolve the sample in pyridine (1.0 ml) containing hydroxylamine hydrochloride (925 mg/ml) and phenyl-β-D-glucopyranoside (1 to 5 mg/ml) as the internal standard. React the solution at 70°C for 30 min. Cool and proceed as in step 4 of sample preparation and derivatization.
1.5.4.2.3 Determination of Mono- and Disaccharides as O-Acetyl Derivatives

This method includes the optional step of reduction to alditols prior to acetylation.

Sample preparation and derivatization:

1. Either extract the saccharides and clean up the extract to remove acids and proteins (see Section 1.5.2.1) or, for the identification or determination of polysaccharides, isolate the polysaccharide and hydrolyze by acid treatment.

2. Take a sample ideally equivalent to at least 5 mg saccharides in a 1.0-ml solution, and add 0.5 ml internal standard solution (allose or inositol at 2 mg/ml).

3. Add 0.4 ml 12.5-M ammonia solution and 0.1 ml sodium tetrahydroborate solution, and react for 30 min at 40°C to reduce aldoses to alditols. Add 0.2 ml acetic acid.

4. Take 0.5 ml solution from step 2 or 3, add 0.5 ml N-methylimidazole and 5.0 ml acetic anhydride, and react for 10 min.

5. Add 0.2 ml ethanol and leave for 5 min.

6. Add 2 x 5.0 ml 7.5-M potassium hydroxide solution, mixing thoroughly each time.

7. Remove the ethyl acetate (top) layer for GLC analysis.

Gas chromatography operating conditions:

1. Column — 25 m x 0.32 m x 0.25 µm film, Rtx-2330 (or similar polar column)

2. Carrier gas — Hydrogen or helium at 15 psi (gas velocity of 60 or 40 cm/s, respectively)*

3. Injection — Split-mode operation, split vent 25 ml/min, 1 µl injection at 250°C

4. Detector — Flame ionization detector at 250°C

5. Oven temperature — 160°C for 1 min, ramp to 240°C at 4 deg/min

 Calibration and quantification:

1. Prepare calibration standards by taking aliquots of aqueous solutions of reference standards equivalent to 1 mg each saccharide and proceed as in steps 2 to 7 for sample preparation and derivatization, above.

2. Determine response factors relative to the internal standard by analysis, as in the gas chromatography operating conditions, above.

3. Quantify the amounts of each mono- and disaccharide in the sample from the peak area data and the response factors.

* This is approximately twice the optimum gas velocity to reduce the analysis time. As an alternative, a 10- to 15-m column could be used at optimum gas velocity and similar performance achieved.
1.6 HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC DETERMINATION OF MONO- AND DISACCHARIDES

High-performance liquid chromatography has become today the method of choice for most types of sugar analysis, from routine determination of common sugars in foods to more demanding investigations requiring both the separation of complex mixtures and high sensitivity. The past several decades have seen major developments in both column and detector technology, providing the analyst with a range of options. The following review of HPLC techniques is intended to be a practical guide to selecting hardware and methodology for sugar analysis according to the required application and is not an exhaustive survey of the literature. The hardware is considered first and has been organized into column choice and detector options. After the survey of methods available for the end determination, sample preparation requirements are considered next. Finally, three outline procedures are presented that illustrate common practice in sugar analysis and should provide sufficient information for the analyst to reproduce the determination.

1.6.1 HPLC Columns

Schwarzenbach [6] introduced the amino-bonded silica column in 1976. The Aminex range of fixed-ion resin columns (Bio-Rad Laboratories) followed in the early 1980s, and later Dionex introduced anion-exchange HPLC columns. These three techniques, discussed below, represent the principal HPLC approaches to sugar separations in common use.

1.6.1.1 Amino-Bonded and Amine-Modified Silica Columns

Aminopropyl-bonded silica represents one of the most widely used stationary phases for the analysis of food sugars. Separation of the common food sugars is readily achieved in around 10 min by isocratic elution with 70 to 80% aqueous acetonitrile (Figure 1.8 and Figure 1.9). Resolution can be controlled by changing the proportion of water and acetonitrile; the speed of elution is increased with increasing water content. A drawback to the use of this phase is the gradual loss of the aminopropyl groups by hydrolysis and by condensation reactions with aldehydes in the food extract to form Schiff’s bases [29]. The resulting reduction in retention can be counteracted by reducing the water content of the mobile phase to slow elution. The most critical separation on this column is that between the early eluting pair fructose and glucose. The lifetime of the column is determined by the point when the required resolution of these two peaks can no longer be maintained through changing the mobile phase composition and becomes unacceptable. An AOAC method for sugar
analysis on aminopropyl columns [30] defines a minimum resolution of 1.0 for the fructose and glucose peaks. Routine measurement of resolution factors in this way allows control of column separation performance.

Column life can be maximized by keeping the water content of the mobile phase down to around 20% while maintaining a reasonable run time by using a fast flow rate. A guard column in series with the analytical column will also help to extend column lifetime by protecting the column from components in the sample extract that might form Schiff’s bases or cause hydrolysis of the aminopropyl groups.

An interlaboratory study was conducted on the determination of mono- and disaccharides using amino columns and refractive index detection [31]. The foods analyzed included dairy products, cereal products, soft drinks, and confectionery and chocolate products. The authors concluded that HPLC can provide an acceptable precision even when different equipment is used and the sample matrix is complex.

Oligosaccharides may also be separated on this column, usually by using a higher water content mobile phase [32]. Jeon and Saunders [33] analyzed
mixtures of glucose, galactose, lactose, and other di- and trisaccharides using 75:25 acetonitrile water. Glucose and galactose coeluted.

Amine-modified silicas can be generated in situ by using a nonbonded silica column and adding a suitable polyamine such as tetraethylene pentamine to the acetonitrile/water mobile phase [34]. Separations comparable to aminopropyl-bonded columns are achieved. The advantage of this approach over aminopropyl columns is that loss of amino groups is not a problem, as the amine bound to the silica surface is constantly being replaced. In contrast to aminopropyl columns, sugar retention behavior can be altered to some extent by the type and concentration of modifier. For example, using the proprietary modifier SAM II offers better separation of the critical pairs glucose/galactose and xylose/arabinose than SAM I [35]. The disadvantage of this approach is that an equilibration time of several hours is required to form a stable column.

In the analysis of food extracts, two separation problems may be encountered with aminopropyl and amine-modified columns. First, products containing
sorbitol, either naturally (e.g., in fruit juices) or as a sweetener (e.g., in preserves and confectionery), present a problem because of the coelution of sorbitol and glucose. Slowing the elution of the sugars by using a low-water-content mobile phase and longer columns can give acceptable separation. Shaw and Wilson [36] separated sorbitol in apple and pear juices using an amino column and an acetonitrile–water mixture of 92:8. Brando et al. [37] used an amino column and a cyanopropyl/amine column in series to separate sorbitol with a mobile phase of 80:15:5 acetonitrile–water–ethanol. Alternative chromatographic approaches that fully resolve sorbitol (e.g., fixed-ion or anion-exchange chromatography) are generally preferred for determining sorbitol.

The second common problem likely to be encountered again involves coelution with glucose, but this time with sodium chloride. Salt is often found in significant concentrations in processed foods, and strategies for removing this interference are needed. Sodium chloride and other salts may be removed at the sample preparation stage by a deionizing treatment. This is discussed later in the section on sample preparation. As an alternative, the chromatography can be modified to displace the salt peak so it no longer coelutes with sugars of interest. De Vries et al. [38] recommended washing aminopropyl–silica columns with a solution of the amine modifier tetraethylene pentamine (TEPA) in the mobile phase (pH adjusted to 7). This results in a shift in the salt peak so it no longer overlaps with the glucose peak but instead runs nearer the solvent front. In the authors’ experience, the conditioning effect is short lived, the TEPA being relatively quickly washed from the column. Running with a mobile phase containing a low concentration of TEPA (pH adjusted to 7 with acetic acid) maintains the effect; however, as has been observed with amine-modified silica columns [39], injections of sample solutions richer in water content than the amine-containing mobile phase generates some late eluting artifact peaks. Analysis run times may have to be extended by up to 1 hr. These peaks may be minimized by careful adjustment of the mobile phase pH. Hendrix et al. [39] found a pH of 8.9 to be optimum using a silica column and 0.02% TEPA.

Acetonitrile is a relatively expensive solvent, and its use for chromatography on both amino-bonded and amine-modified columns adds to the costs of routine analysis. Solvent usage and, therefore, cost can be minimized in two ways. Mobile phase cyclers are now commercially available. These return mobile phase eluent to the reservoir when no components are eluting. The switching times can be simply timed from the point of injection or triggered by the detector response to eluting sugars. A simple way of reducing solvent consumption is to use a narrow-bore column. Moving from a standard 4.6 × 250-mm configuration to a midbore 3.2 × 250-mm column requires a halving of the flow rate (ml/min) to maintain the same linear velocity (cm/min); hence, an immediate halving of solvent consumption is achieved. Unlike true narrow-bore columns of 2.2-mm internal diameter (i.d.) which generally require detector flow cells and injectors optimized to suit the smaller column capacity,
the only modification that may be required for changing to a midbore column is the use of a smaller injection volume. In the authors’ laboratory, a reduction in loop size from 20 µl to 10 µl resulted in no significant change in peak height. This is because the narrower column has a higher efficiency, resulting in a smaller peak width and an increase in peak height.

1.6.1.2 Amino-Bonded Resin Columns

Another development in column technology is the introduction of column supports based on a backbone of vinyl alcohol copolymers. This hard gel polymer base is analogous to the silica backbone of conventional bonded phase columns, and a range of bonding chemistries is available, including amino-bonded columns. The AsahipaK-NH2P column (Showa Denko America, Inc., New York) offers separation performance similar to that of silica-based products but with the advantage of greater durability. The column packings have a wide pH stability range and hence are more resistant to hydrolytic deterioration than silica packings. The only drawback is the significantly greater cost of these columns, although this might be justified by the greater reproducibility and longer lifetime.

1.6.1.3 Fixed-Ion Resin Columns

Fixed-ion resin columns are commonly used for the analysis of both simple mono- and disaccharides as well as oligosaccharides. A strong cation-exchanging resin based on a cross-linked styrene–divinylbenzene copolymer is used. This may be prepared in different ionic forms by treating the protonated form of the resin with, typically, calcium, sodium, lead, or silver salts [40]. The mode of separation is a combination of ligand exchange, size exclusion, and reverse-phase partition. Aminex resin columns were originally developed by Bio-Rad Laboratories (Richmond, CA).

Water is commonly used as the mobile phase, although a very dilute salt solution of the same cation type may also be employed. The columns are operated at elevated temperatures (75 to 85°C is typical) to lower viscosity and speed diffusion within the resin bed, thereby improving efficiency and reducing back pressure. At lower temperatures, double peaks of the different sugar anomers occur, but they fuse at high temperatures due to the increased mutarotation rate. The glucose peak may appear broader than, for example, the sucrose peak for this reason. The resins are sensitive to high pressures, collapsing at pressures over 1000 to 1500 psi, so column back pressure must be minimized. This is the reason for the typical column bore of 7.8 mm. These columns do not suffer from the deterioration in the stationary phase and performance experienced with aminopropyl columns and, with care, can have a long lifetime. Contaminants can often be removed by extensive washing with water, and any loss of cation can be rectified by washing with a salt
solution of the appropriate cation type. The main requirement for maximizing column life is to keep particulates from getting onto the column by ensuring adequate filtering of sample extracts, ideally using a 0.2-µm membrane filter.

On fixed-ion columns, oligosaccharides elute first, followed by disaccharides and then monosaccharides — the reverse order of aminopropyl/amine-modified columns. Sugar alcohols such as sorbitol and mannitol elute latest, thus they are well resolved from other sugars. Advantages of this technique, then, are the inexpensive eluent, high column capacity and stability, and complete elution of all sugars injected.

With such a simple mobile phase there is little scope for modifying the resolution of the column; however, the separation of particular sugar mixtures can be optimized by altering the cation type, the degree of cross-linking of the resin, and the reverse-phase character of the resin backbone. Selectivity for a particular separation may thus be built into the column.

The most commonly used column type is the calcium ionic form. Using an 8% cross-linked resin, class separation of di-, tri-, and tetrasaccharides is routinely achieved. Complete separation of all the simple sugars is not possible, so, while glucose and fructose can be fully resolved, maltose and sucrose coelute. This limits the applications of this technique for general food analysis to simple sugar mixtures such as fruit juices containing sucrose but not maltose (Figure 1.10) and sugar syrups that contain maltose but not sucrose (Figure 1.11). For both these examples, which contain little in the way of potential column contaminants, the column provides a good, long-life alternative to aminopropyl/amine-modified columns.

The sodium-form column is useful for sample extracts that contain high concentrations of inorganic salts that foul columns of other ionic forms. They are easily regenerated to their original ionic state.

The lead-form column provides the best separation of monosaccharides and will separate the neutral sugars: glucose, xylose, galactose, arabinose, and mannose. Slavin and Marlett [41] used this column to analyze dietary fiber hydrolysates by separating the above sugars, although they found that rhamnose and galactose coeluted. The same authors showed that an amino column produced inadequate separation of the hexoses mannose, glucose, and galactose.

Even the hydrogen-form column may be used for sugar separations. This column is more usually employed for the analysis of organic acids using a dilute acid as the mobile phase. The simultaneous determination of acids and sugars using ultraviolet (UV) and refractive index (RI) detection, respectively, has been reported by Lazaro et al. [42].

1.6.1.4 Anion-Exchange Resin Columns

Anion-exchange chromatography has grown in importance as a valuable technique for the analysis of both simple and complex sugar mixtures. The reasons for this are twofold. First, this mode of separation provides greater scope for
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the separation of saccharides (simple sugars and oligosaccharides) than the techniques so far discussed. Second, the coupling of this technique with pulsed-amperometric detection, as is normal practice, gives a tremendous advantage over RI or UV detection systems. Because it is highly specific for carbohydrates, interferences are largely removed (some interferences from amines and certain sulfur species are possible), and, together with its high sensitivity, the two main disadvantages of the RI detector are overcome. The pulsed-amperometric detector is discussed in the following section.

Anion-exchange chromatography of sugars involves the use of a mobile phase pH between 11 and 13 (1- to 150-mM NaOH), which induces ionization of the sugar hydroxyl groups. Separation occurs by differential retention of the ionized sugars on the anion-exchange resin surface. Retention increases with the number of hydroxyl groups per molecule so the elution order is generally mono-, di-, and oligosaccharides. Different hydroxyl groups within a particular sugar molecule may have different ionization potentials; thus, by altering the molarity of the alkali in the mobile phase the degree of ionization can be altered for a particular sugar. By turning hydroxyl groups “on” or “off” in this way, the degree of ionization of a particular sugar can be altered and thereby its degree of retention on the column.

FIGURE 1.10 Analysis of apple juice sugars. Column, Resex RCM-monosaccharide (Phenomenex), 300 mm × 7.8 mm; mobile phase, water, 85°C, 0.6 ml/min; refractive index detection. 1, Sucrose; 2, glucose; 3, fructose; 4, sorbitol.
The retention behavior of sugars also depends on other factors, such as molecular size and steric orientation of the ionized hydroxyl groups, providing even more options for the separation of sugar mixtures. Sugar resolution can be further modified by the addition of low concentrations of a zinc salt (typically 0.2- to 0.3-mM zinc). Acetate is used to improve the separation of certain mono- and disaccharide mixtures.

Although retention may be altered by changing the hydroxide concentration, more usually a competitor ion mechanism is used. Acetate ions added to the mobile phase compete with sugar molecules for the resin-binding sites, and sugar retention is thereby lowered. Acetate gradients are common practice with this technique and are made possible by the use of pulsed-amperometric detection, which is not significantly affected by the change in acetate concentration. Acetate gradients allow the separation of mono-, di-, and oligosaccharides in one analysis and within a reasonable run time. Olieman [43] used a ternary gradient of hydroxide and acetate to determine monosaccharides, disaccharides, and maltodextrins in a single analysis and studied fruit yogurt, baby food, and confectionery.

Columns with different retention characteristics offer further scope for achieving optimum separation of different sugar mixtures. For separations of mono- and disaccharides, the CarboPac PA-1 (Dionex Corporation; Sunnyvale, CA) offers good retention and resolution [44]. Corradini [45] separated sorbitol,
fructose, glucose, and sucrose in fruit juices. Typical separations are illustrated in Figure 1.12 and Figure 1.13. This column is generally less suited to oligosaccharide analysis because of the long retention times obtained. The CarboPac PA-100 column has been optimized for this purpose and still provides reasonable separation of the common food sugars. Finally, the CarboPac MA1 has been developed to provide greater retention and thereby resolution of sugar alcohols (e.g., sorbitol, mannitol) which elute early on PA1 and PA100 columns and are therefore prone to interferences.

The full separating power of anion-exchange chromatography is demonstrated by its ability to resolve complex mixtures of mono- and disaccharides. Prodollet et al. [46] reported the separation in a single run of fucose, rhamnose, arabinose, galactose, glucose, sucrose, xylose, mannose, fructose, and ribose in soluble coffee solids. The neutral sugars making up dietary fiber hydrolysates represent a difficult separation that has been optimized by Quigley and Englyst [9,47]. Fucose, rhamnose, arabinose, galactose, glucose, xylose, and mannose are resolved in a separation that cannot be achieved using amino-propyl/amine-modified columns or fixed-ion resin columns. GLC determination is the only other alternative for this analysis [9].
1.6.2 HPLC Detectors

During the early development of HPLC for sugar analysis, RI and, to a lesser extent, UV detectors were used almost exclusively. Steady improvement in detector design and the introduction of alternative modes of detection have resulted in a much wider range of options. These are now discussed.

1.6.2.1 Refractive Index Detectors

Refractive index detectors for HPLC analysis work on the principle of differential refractometry and are typically of the deflection type. The flow cell is divided into a reference liquid compartment and a sample liquid compartment. A light beam passing between these two compartments is deflected in proportion to the difference in refractive index between the reference and sample liquids. The deflection is measured by the displacement on a light-receiving element. Modern instruments are very much improved over instruments available in the past, offering much lower noise and better baseline stability as well as greater sensitivity. This has been achieved by improved optical and electrical...
systems and better temperature stability. Refractive index is temperature dependent, and a thermostated flow cell and liquid lines are necessary for good baseline stability. Because of the effect of small changes in temperature on the baseline, it is normally advisable also to thermostat the HPLC column using a column oven (typically set at 35°C).

The analysis of common sugars in foods normally does not require high sensitivity. Individual sugar concentrations down to 0.05% may be measured in the routine analysis of foods, and this is usually adequate. If greater sensitivity is required, alternative detectors must be used.

The refractive index detector is often referred to as a “universal” detector because of its nonselective response; however, this lack of specificity can be a disadvantage when other significant components of the food matrix interfere in the separation of the sugar profile. The limits of detection quoted above may then be more difficult to achieve.

Another disadvantage of the refractive index detector is its incompatibility with gradient operation. Such changes in mobile phase composition generally result in an unacceptably steep baseline change. Analysis of sugar mixtures requiring different elution conditions must therefore be conducted using more than one analytical run.

Despite these disadvantages, the refractive index detector remains a good, low-cost choice for the routine analysis of common food sugars.

### 1.6.2.2 Ultraviolet Detectors

Sugars may be detected by ultraviolet spectrophotometry either directly or as UV-absorbing derivatives. Direct detection is possible by monitoring at a wavelength below 200 nm; however, at this wavelength problems are likely to be encountered with solvent purity and interferences, so this is not a practical approach for routine use. Derivatization of sugars to form strong UV-absorbing chromophores allows the problem of interferences to be reduced and at the same time provides enhanced sensitivity over RI detection; however, artifacts formed by the derivatization process may give rise to interferences. A variety of derivatives have been used. Perbenzoates [48] and 4-nitrobenzoates [49] have been prepared, but these give rise to several anomic derivatives. A single peak for each mono- and disaccharide may be obtained using the benzoyloxime–perbenzoyl derivatives [50]. These can be determined at the nanogram level.

### 1.6.2.3 Pulsed-Amperometric Detectors

Carbohydrates can be oxidized at a gold or platinum electrode giving a high initial current [51]; however, as an oxide layer forms and the products of oxidation rapidly coat and poison the electrode surface, further oxidation is inhibited, causing the current to decay rapidly. By rapidly pulsing between
high positive and negative potentials, the electrode surface is cleaned and reactivated (oxide layer reduced) between measurement of the carbohydrate oxidization current.

Pulsed-amperometric detection (PAD) provides highly specific detection of carbohydrates and, at the same time, high sensitivity. Limits of detection for glucose and sorbitol of 30 ppb have been reported [51]. Sample preparation is simplified when using this method of detection because non-sugar coextractives do not interfere and clean-up procedures are generally not required. Removal of some potential column contaminants may, however, be advisable (e.g., high protein levels, halides, sulfate).

A high pH (above 11) is necessary for the detector to function correctly. This will generally be provided by the mobile phase when using anion-exchange chromatography; however, if the separation requires the use of a weakly alkali mobile phase, the necessary alkali strength at the detector may be provided by the postcolumn addition of, typically, 0.3-M NaOH at 0.5 ml/min. Postcolumn addition is also used when hydroxide gradients are required and gives a stable baseline, unaffected by the changing alkalinity of the mobile phase.

Because significant changes in pH will affect the detector response and cause baseline shifts it is usual practice in anion-exchange chromatography with gradient operation to maintain a fixed hydroxide concentration while changing the acetate concentration. In some applications requiring both a changing hydroxide and acetate gradient, a pH counter electrode may be used to replace the standard Ag/AgCl reference electrode.

1.6.2.4 Evaporative Light-Scattering Detectors

The evaporative light-scattering detector (ELSD) developed out of the need for a universal HPLC detector that, unlike RI detectors, could achieve high sensitivity and would be compatible with mobile phase gradient operation. The principle of detection is simple. Column eluent is atomized in a heated air or nitrogen stream. Mobile phase solvents (including water) are vaporized, and any nonvolatile components are left as a fine mist of particles that then passes through a light beam (modern instruments use a laser light source). Light scattered by the particles is detected by a photomultiplier positioned at an angle of 120° to the light beam. The intensity of the scattered light is proportional to the number of particles and the amount of material. The response is not linear over a wide concentration range, although a linear region of operation is usually possible. It may be necessary to produce a logarithmic calibration curve. The detector requires a high flow rate of air or nitrogen (the latter being advisable when using inflammable solvents) and a fume hood or extraction facility to handle the solvent vapors (essential if one is using acetonitrile).
Macrae and Dick [32] described an early use of the ELSD for sugar analysis. Using an aminopropyl column and a gradient of 20 to 30% water in acetonitrile, they separated sucrose, raffinose, and stachyose in a soya extract within 20 min. Morin-Allory and Herbreteau [52] used a light-scattering detector to demonstrate the use of supercritical fluid chromatography to analyze a range of monosaccharides and polyols. Clement et al. [53] recently compared ELSD and RI detection for the analysis of a range of mono- and disaccharides and concluded that the ELSD offers better sensitivity and a more stable baseline than an RI detector and it makes gradient elution possible. Although it overcomes many of the disadvantages of the RI detector, the ELSD is not selective like the PAD, so coextractives can still interfere.

As a universal detector, the ELSD is very powerful. For example, it is the detector of choice for lipid analysis and, if other such applications are required in the food analysis laboratory, it might well be considered as an alternative to the RI detector for use in sugar analysis.

1.6.3 Sample Preparation for HPLC Analysis

Sample preparation at its simplest involves a straightforward aqueous extraction of the sample; however, an aqueous extraction may extract numerous polar components other than sugars, and these may interfere in the HPLC analysis, particularly when one is using RI detection. These compounds may also contaminate the column and reduce its useful lifetime; as a consequence, sample preparation often involves further cleanup of the extract prior to analysis.

Extraction is facilitated by heating, but care needs to be taken to avoid inversion of sucrose to fructose and glucose. Inversion is catalyzed in an acid solution; therefore, particular care is required with acidic samples. Chutneys are a good example of a high-sugar/high-acid food susceptible to inversion.

A procedure used in the authors’ laboratory is an aqueous extraction at 65 to 70°C, followed by dilution with acetonitrile and filtration prior to analysis. Acetonitrile addition serves several purposes. First, it is necessary for compatibility with the HPLC system being used — separation on an aminopropyl–silica column with acetonitrile–water. Water is a strong eluent with this system, and a fully aqueous sample solvent may tend to “push” the injected sugars, resulting in peak fronting effects and poor resolution, particularly of early eluting peaks.

Acetonitrile dilution has the additional benefit of precipitating potential interferences such as proteins and polysaccharides. Care must be taken to avoid partially precipitating sugars of low solubility; for example, lactose occurring in milk products is not soluble in acetonitrile–water mixtures containing less than 15% water.

Yet another benefit of adding acetonitrile is deactivation of any enzymes that might change the sugar composition. Sample extracts and sugar standards
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Prepared in aqueous acetonitrile can usually be stored for several months at refrigeration temperatures without signs of change. Aqueous sugar solutions, on the other hand, may suffer decomposition on standing due to microbial contamination.

An alternative to the precipitation approach is to use a more selective extracting solution that will dissolve simple sugars but not starches, proteins, and other high-molecular-weight material. Aqueous ethanol (80%) is commonly used, usually with heating, for example, under reflux. Again, care must be exercised to ensure that all of the sugars of interest are sufficiently soluble and that heating does not result in sucrose inversion.

Whether one is using selective extraction or precipitation techniques, further clean-up of the extract may be necessary. If alcoholic extraction is used, any lipid present may be coextracted. This may be removed by extraction into an immiscible solvent such as chloroform. As an alternative, the sample may be defatted prior to extraction of the sugars. For solid samples, this is readily achieved by warming with hexane or petroleum ether, centrifuging, and decanting the lipid-containing solvent. For samples with a moderate to high fat content, defatting is an essential pretreatment for efficient extraction as it improves contact between sugars in the sample and the aqueous extracting solution.

Large soluble molecules such as polysaccharides and proteins can be removed by coprecipitation with Carrez solution. This treatment is particularly important if the sample is enzyme active; for example, uncooked flour may contain active β-amylase, which, in a warmed aqueous solution, will break down starch polysaccharides to maltose. Treatment with Carrez reagent will remove soluble polysaccharide substrates for β-amylase, but the reagent must be added as soon as possible to the extract and certainly before heating. The alternative to coprecipitation techniques is to add sodium azide or silver nitrate to inactivate any enzymes present.

Salt is a major interference in the HPLC separation of sugars, as mentioned earlier with regard to aminopropyl–silica columns. One strategy that may be employed with these columns is to use an amine-modifier such as tetraethylene pentamine to shift the salt peak so it no longer overlaps with the fructose or glucose peaks but instead runs nearer the solvent front [38]. Salt interference is also a problem on fixed-ion resin columns when one is using RI detection for which there is no chromatographic solution. Sodium salts will also tend to displace calcium ions from a calcium-form column.

It is usually better practice to remove interfering salts by an ion-exchange clean-up; this procedure will often remove not only interferences but also, in the case of both aminopropyl columns and fixed-ion resin columns, compounds that will cause deterioration of the column.

Ion-exchange resins have been widely used for deionizing samples, particularly wines and fruit juices, to remove amino acids and organic acids. Self-
packed columns are often used, and after a sample extract is eluted the resin can be cleaned and regenerated. Both cation- and anion-exchange resins can be used. In a typical case, an aqueous extract might be passed through 5-cm$^3$ AG 50W-X8 cation-exchange resin (Bio-Rad Laboratories) or 5-cm$^3$ AGI-S4 anion-exchange resin (Bio-Rad Laboratories). Disposable cartridges are also available.

Silica-based ion exchangers are a less expensive alternative to resins. Both weak anion-exchange and weak cation-exchange forms are available based on sulfonic acid and quaternary ammonium salt derivatives, respectively. They are available as disposable solid-phase extraction cartridges in different formats of packing quantity and reservoir size. The standard format — 500 mg adsorbent and a 3-ml reservoir capacity — will be sufficient for removal of low to moderate levels of ionic contaminants.

The usual procedure is to push the sample extract through a preconditioned cartridge, discarding the first few drops before collecting the eluent. The flow rate through the cartridge should be controlled to ensure repeatability, and sugar recovery should be routinely checked using either a standard solution or, preferably, a control sample extract.

Solid-phase extraction cartridges may also be used to remove nonpolar interferences. SepPak or other C18 silica-based products can be used for this purpose.

1.6.4 Applications and Practical Procedures for HPLC Analysis

The preceding discussion of column types and detectors has demonstrated the potential of the systems available. The analysts’ choice depends on a number of factors, including the sugars of interest, the sample matrix, and, very importantly, the cost of the equipment. For determination of the common food sugars in a wide range of food matrices (the norm for a general food analysis laboratory), an aminopropyl–silica column and RI detection are adequate. Besides the detector, equipment requirements include a basic isocratic pump and a column oven. The amino column may be substituted or supplemented by a calcium-form resin column. This would be an appropriate choice for the analysis of fruit juices or sugar syrups and would also permit the determination of glucose oligomers in foods. A more expensive option is anion-exchange chromatography with PAD detection. If the additional cost can be justified, this approach will certainly provide better sensitivity and data less prone to the errors of coextractive interferences. Sample preparation time is also likely to be shorter because extensive clean-up is often not required.

The following procedures have been chosen to illustrate the sample preparation techniques and chromatographic techniques described above, and they represent common practice in the food analysis laboratory.
1.6.4.1 Determination of Mono- and Disaccharides on an Aminopropyl-Silica Column with RI Detection

1.6.4.1.1 Sample Preparation
Weigh a 1- to 2-g sample into a 30-ml screw-capped bottle. Defat if necessary by warming with hexane, centrifuging, decanting hexane, and evaporating on a steam bath and in an oven. Add 15 ml water (or, if necessary, 1 ml Carrez A, 1 ml Carrez B, and 13 ml water), cap, shake, and heat for 30 min in an oven at 65 to 70°C. Cool, add 10 ml acetonitrile, mix, and filter through glass fiber paper. If required, pass the extract (2.5 ml) through a cation-exchange cartridge, eluting 1.25 ml to waste and collecting the remainder for analysis.

1.6.4.1.2 Preparation of Standards
Prepare aqueous solutions containing the sugars of interest — fructose, glucose, sucrose, maltose, and lactose. Mix these with acetonitrile in the proportion 60:40. Do not make up to volume if equivalence with the sample preparation procedure above is to be maintained. (Note: 60 ml water and 40 ml acetonitrile mixed together give a total volume less than 100 ml.) The final concentration of each sugar should be in the range of 0.5 to 3.0 mg/ml.

1.6.4.1.3 HPLC Conditions
- Column — 250 mm × 4.6 mm (or 3.2 mm) aminopropyl-silica (e.g., Spherisorb NH2 or Hypersil NH2) thermostated at 35°C
- Mobile phase — Acetonitrile–water, 75:25 to 85:15, depending on age of the column
- Flow rate — 1.8 ml/min (or 0.9 ml/min for 3.2-mm column)
- Injection volume — 10 to 20 µl
- RI detection — Thermostated at 35°C

1.6.4.2 Determination of Mono- and Disaccharides by Anion-Exchange Chromatography with Pulsed-Amperometric Detection

1.6.4.2.1 Sample Preparation
Dilute 1- to 2-g sample or 1 to 5-ml liquid sample to 100 ml with water (add 1 ml each of Carrez A and Carrez B and/or up to 50 ml acetonitrile prior to dilution as necessary). Dilute with water to bring sugar concentrations into calibration range (e.g., 10-fold), and filter through a 0.2-µm membrane filter.

1.6.4.2.2 Preparation of Standards
Prepare aqueous solutions containing 10 to 100 ppm of each sugar.
1.6.4.2.3  **HPLC Conditions**

- **Column** — 250 × 4-mm CarboPac PA1 + 50 × 4-mm CarboPac PA1 guard column
- **Mobile phase** — 150-mM NaOH (prepared by adding 50% m/v NaOH to helium-degassed water)
- **Flow rate** — 1.0 ml/min
- **Injection volume** — 20 to 50 µl
- **Detector** — PAD with gold electrode

1.6.4.3  **Determination of Sorbitol and Mono- and Disaccharides on a Fixed-Ion Resin Column with RI Detection**

1.6.4.3.1  **Sample Preparation**

Prepare syrup (0.25 g) dissolved in 25 ml water and fruit juice (5 ml) diluted to 100 ml. Samples of other foods (1 to 2 g) may be prepared as in Section 1.6.4.1 above, substituting water for acetonitrile. All sample extracts should be filtered through 0.2-µm membrane filters.

1.6.4.3.1  **Preparation of Standards**

Prepare aqueous solutions containing sucrose, glucose, fructose, and sorbitol as required at 1 to 4 mg/ml. The calibration factors for glucose may be used for maltose and higher glucose oligomers.

1.6.4.3.3  **HPLC Conditions**

- **Column** — 300 × 7.8-mm Aminex HPX-87C column (calcium form) or alternative (e.g., Resex RCM-monosaccharide, calcium form) thermostated at 85°C
- **Mobile phase** — Water (preheated to 85°C by passing tubing through column oven)
- **Flow rate** — 0.4 ml/min (mono- and disaccharides only and glucose oligomers) or 0.6 ml/min (mono- and disaccharides only and/or sorbitol)
- **Detection** — Refractive index

1.7  **SUMMARY AND CONCLUSIONS**

The preceding sections indicate that the carbohydrate analyst has a wide range of options for the determination of mono- and disaccharides in foods. The current state of chromatographic techniques can provide powerful and versatile analytical methods allowing for both selective and sensitive determination. The approach selected may well be dependent on the chromatographic equipment available in the food analysis laboratory. Gas chromatographic determination...
requires only a basic instrument and flame ionization detection. For routine high-performance liquid chromatography determination of common food sugars, a simple isocratic system incorporating an aminopropyl–silica column and a refractive index detector is adequate. The range of analyses can be extended by the use of a fixed-ion resin column. An alternative detector is the evaporative light-scattering detector, which provides greater sensitivity and the potential for use in more complex separations requiring gradient elution. Where high sensitivity and selectivity are demanded by the analysis, a more sophisticated HPLC system based on anion-exchange chromatography and pulsed amperometric detection is likely to be the best solution. The analyst must give serious consideration to all the determinations that may be required before deciding on the analytical strategy and selecting the chromatography system. The actual procedures adopted for optimum quantitative determinations are very dependent on the mono- and disaccharides to be determined and the nature of the samples to be analyzed.

REFERENCES


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2 Mono- and Disaccharides: Selected Physicochemical and Functional Aspects

Kirs Jouppila

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2.1 INTRODUCTION

Mono- and disaccharides are sugars containing carbon, oxygen, and hydrogen atoms, and they are classified as carbohydrates, which also include oligo- and polysaccharides. Mono- and disaccharides are the lowest molecular weight carbohydrates. They are formed in plants [1], and they can be separated from plant material using, for example, water extraction followed by crystallization [2]. Mono- and disaccharides are often stored in stable crystal form. They are added to foods to increase sweetness, to give color and flavor (as a result of nonenzymatic browning and caramellization reactions), and to increase storage stability by lowering the water activity (a_w) of a product. The physicochemical and functional properties (sweetness, solubility, melting temperature, glass transition temperature, and reactivity) of mono- and disaccharides differ, although the molecular structures are quite similar.

Mono- and disaccharides may exist in amorphous form with a random molecular order in some food products, as reviewed by White and Cakebread [3] and Roos [4]. Such products include dehydrated food products, such as milk and whey powders, that are obtained via spray-drying, as well as freeze-dried fruit and berries and hard sugar candies that have been produced by rapid cooling. Also, other food processes, such as freezing and extrusion cooking, can produce amorphous (noncrystalline), often solid structures, such as a freeze-concentrated, unfrozen phase of frozen products (e.g., in ice cream) and crisp and brittle snack products, respectively. Amorphous sugar and a sugar-containing matrix may encapsulate various compounds, such as aroma compounds and bioactive substances, as reviewed by Karel [5]. Encapsulation may increase the stability of these compounds by preventing oxidation of the encapsulated compounds.

Amorphous food materials are in a glassy, metastable state if they are stored at temperatures lower than their glass transition temperature [3,4,6]. Water plasticizes amorphous food materials, resulting in lowered glass transition temperatures. If the glass transition temperature is lower than the ambient temperature, molecular mobility in the material increases, and various changes may occur during food processing and storage. Stickiness and caking are desired phenomena in the agglomeration process but are undesired changes during the storage of food powders. Crystallization of sugars is often an undesired phenomenon during storage; for example, crystallization of lactose can decrease the quality of dairy powders because of the poor solubility of crystalline lactose and affects the quality of ice cream because crystallized lactose gives an unpleasant, sandy mouthfeel [3]. Crystallizing, however, is the main unit operation for producing crystalline sugars, so controlling crystallization might involve either preventing crystallization or promoting crystal formation and growth, as reviewed by Hartel [7,8]. Water sorption and water plasticization data for amorphous sugars and sugar-containing products are necessary to predict the occurrence and rate of various potential changes during processing and storage [4,6].

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This chapter discusses the selected physicochemical and functional aspects of mono- and disaccharides, with an emphasis on amorphous solid states of sugars and plasticization of amorphous sugars in relation to time-dependent changes, mainly crystallization.

2.2 MOLECULAR STRUCTURE OF MONO- AND DISACCHARIDES

Monosaccharides are the simplest form of sugars with a molecular formula of \( C_n(H_2O)_n \), where \( n \) ranges from 3 to 9 [9,10]. The most common monosaccharides are hexoses, such as \( \alpha \)-glucose and \( \alpha \)-fructose, and pentoses, such as \( \alpha \)-arabinose and \( \alpha \)-arabinose [1]. Monosaccharides having the same molecular weight but different molecular structures, as well as different chemical and physical properties, are isomers to each other. The two fundamental types of isomers are structural and spatial, both of which occur in monosaccharides [1].

Structural isomers of monosaccharides may be functional group isomers: They contain either an aldehyde or a ketone group in their molecular structure and are classified as aldoses (e.g., aldohexose) or ketoses (e.g., hexulose), respectively [1,10]. Structural isomers of monosaccharides may exist in ring forms of different sizes: a five-membered furanose (furan-like) ring or a six-membered pyranose (pyran-like) ring resulting from formation of intramolecular hemiacetals or hemiketals [9]. The carbon atom of the carbonyl group becomes chiral when it is involved in ring formation, resulting in two different anomeric forms of monosaccharide (e.g., \( \alpha \)-\( \alpha \)-glucopyranose, \( \beta \)-\( \beta \)-glucopyranose). Such anomeric forms are also referred to as anomers [1,10]. Mutarotation is the interconversion of these \( \alpha \) and \( \beta \) anomer forms (see Section 2.3.2).

Spatial isomers are also called stereoisomers, which differ from each other in the arrangement of their atoms in space [1]. The two types of stereoisomerism are configurational and conformational isomerism. Configurational stereoisomers of monosaccharides are based on the existence of chiral carbon atoms in their molecular structure. Monosaccharides with three to nine carbon atoms contain one to seven chiral carbon atoms. A chiral, asymmetric carbon atom to which four different groups are attached can exist in two different configurations which are mirror-images of each other [10]. Such configurational stereoisomers, where analogous chiral carbon atoms have the opposite configuration, are referred to as diastereoisomers or epimers [1]. All of the monosaccharides are epimers of other monosaccharides; for example, \( \alpha \)-glucose is a 2-epimer of \( \alpha \)-mannose, a 3-epimer of \( \alpha \)-allose, a 4-epimer of \( \alpha \)-galactose, and a 5-epimer of \( \alpha \)-glucose. For a hexose (such as glucose) containing 4 asymmetric carbon atoms, 16 (= \( 2^4 \)) diastereoisomers are possible, 8 of which belong to a chiral family of \( \alpha \)-sugars and 8 to a chiral family of \( \beta \)-sugars. A sugar belongs to the \( \alpha \) or \( \beta \) family of sugars when its highest numbered chiral carbon atom (carbon 5 in glucose) has the hydroxyl group
written to the right or left, respectively, in the Fischer projection formula [1].

Monosaccharides can also be enantiomers, which are configurational stereoisomers bearing a total mirror-image relation to each other; for example, the enantiomer of D-glucose is L-idose.

Conformational stereoisomerism involves the cyclic forms of monosaccharides [1,10]. For example, β-D-glucopyranose can occur in various conformations, or shapes — two “chair” conformations (with three carbon atoms and the ring oxygen in a plane and two carbon atoms positioned one above and the other below the plane) and various “boat” conformations (with four atoms of the ring in a plane and two atoms of the ring positioned either above or below the plane). Such conformations are energetically favored when bulky groups (hydroxyl and hydroxymethyl groups) are in the equatorial positions, such as the C1 chair conformation of β-D-glucopyranose.

Disaccharides are sugars with a molecular formula of Cn(H2O)n−1. They consist of two monosaccharide units condensed with the concomitant loss of one molecule of water [9]. Disaccharides may be homogeneous, having two similar monosaccharide units such as maltose (4-O-α-D-glucopyranosyl-D-glucopyranose) and α,α-trehalose (α-D-glucopyranosyl-α-D-glucopyranoside), or heterogeneous, having two different monosaccharide units such as lactose (4-O-β-D-galactopyranosyl-D-glucopyranose) and sucrose (α-D-glucopyranosyl-β-D-fructofuranoside) [1]. There are differences in the chemical and physical properties of various disaccharides, although the molecular weights are the same.

Monosaccharides and disaccharides may have a free hemiacetal group in their structure, and such mono- and disaccharides are referred to as reducing sugars [1,9]. In nonreducing disaccharides (e.g., sucrose and α,α-trehalose), both anomic hydroxyl groups participate in the formation of glycosidic linkage between two monosaccharide units. Reducing sugars take part in nonenzymatic browning, which is known as the Maillard reaction or the carbonyl–amine reaction [1]. The relative reactivity of reducing sugars in nonenzymatic browning has been found to increase with decreasing molecular weight. Pentoses are more reactive than hexoses, which are more reactive than reducing disaccharides [1].

2.3 MONO- AND DISACCHARIDES IN WATER SOLUTIONS

2.3.1 SOLUBILITY

According to Hogan and Buckton [11], dissolution involves the disruption of bonding between the solid molecules and the formation of bonds between the solute and the solvent. All the mono- and disaccharides are soluble in water, and most of them have a relatively high degrees of solubility [1]. The solubility
concentration of the solute can be defined as the concentration resulting from maximum solubilization of the solute at a given temperature. In solution at the solubility concentration of the solute, the equilibrium condition between the solid and liquid phases of solute prevails; that is, the chemical potentials of the solute molecules in both liquid and solid phases are equal [8]. The water solubilities of various sugars differ, however, and an increase in temperature results in increased solubilities of various sugars [1,8,12]. The presence of various sugars in solution decreases the solubility of a sugar [1]; for example, in solutions containing both lactose and sucrose, the solubility of lactose was found to decrease with an increasing content of sucrose [13].

Gao and Rytting [14] reported that the heat of solution of crystalline sucrose was 17.3 J g$^{-1}$, whereas that of amorphous, freeze-dried sucrose was –43.4 J g$^{-1}$, determined using solution calorimetry at 25°C. Hogan and Buckton [11] reported corresponding values for crystalline and amorphous lactose; the heat of solution of $\alpha$-lactose monohydrate was 56.2 J g$^{-1}$ whereas that of amorphous, spray-dried lactose was –56.5 J g$^{-1}$. They found that spray-dried lactose dissolved more rapidly in water than $\alpha$-lactose monohydrate when the dissolution of the mixture containing 50% $\alpha$-lactose monohydrate and 50% spray-dried lactose was studied at 25°C.

Gao and Rytting [14] and Hogan and Buckton [11] found that the enthalpy of solution increased linearly with a decreasing content of amorphous sugar and an increasing content of crystalline sugar in the mixture. Hogan and Buckton [11] suggested that solution calorimetry can be used in the quantification of relatively small contents (from 1 to 10%) of amorphous material in predominantly crystalline material assuming that the enthalpies of solution of the amorphous and crystalline forms differ.

### 2.3.2 Mutarotation

Mutarotation of a reducing sugar in solution may involve five structural isomers: $\alpha$- and $\beta$-pyranose, $\alpha$- and $\beta$-furanose, and the aldehydo or keto (open-chain) form [1]; however, the presence of all the various isomers at the same time is uncommon. For example, mutarotation of D-glucose at 20°C results in a mixture containing 36% $\alpha$-D-glucopyranose and 64% $\beta$-D-glucopyranose because of the instability of the furanose forms and the very low concentration of the open-chain form [1,12]. Mutarotation of a reducing sugar in solution can be observed using a polarimeter because mutarotation causes changes in the optical rotatory power due to changes in the amounts of the anomeric forms of sugar [1]. Mutarotation occurs until an equilibrium ratio of anomeric forms is achieved. The rate of mutarotation increases with increasing temperature [1]. According to Hartel [8], the rate of mutarotation is a complex function of solution conditions, such as temperature, concentration, pH, and the presence of impurities.
Specific optical rotations have been reported for anhydrous $\alpha$-lactose and $\beta$-lactose, as well as lactose, in equilibrium solutions at various temperatures [15,16]. The equilibrium ratio of $\beta$ and $\alpha$ anomers of lactose was found to depend on temperature; the ratio of $\beta$ to $\alpha$ decreased with increasing temperature [16]. Also, other solution conditions (e.g., concentration, pH, and the presence of other compounds) have been found to affect the equilibrium ratios of anomeric forms of a reducing sugar [12].

2.3.3 Effect of Mono- and Disaccharides on Colligative Properties

Colligative solution properties are physical properties of solution that change in the presence of solute [17]. Such physical properties include, for example, vapor pressure, freezing point, and boiling point. Mono- and disaccharides affect the colligative properties of water solutions. Water solutions containing mono- or disaccharides have a lower vapor pressure, lower freezing point, and higher boiling point than pure water. According to Chang [18], colligative properties depend only on the number of solute molecules present, not on the molecular weight of solute molecules.

Water solutions containing mono- or disaccharides have a lower vapor pressure and, thus, a lower water activity ($a_w$) than pure water. The lower vapor pressure of a solution is due to a smaller increase in entropy during evaporation of the solvent from a solution than from pure solvent [18]. The water activity is defined as the ratio of the vapor pressure of water (p) exerted by the material to the vapor pressure of pure water ($p_0$) at the same temperature at equilibrium [19]; that is, $a_w = p/p_0$. The water activity of the material can also be determined as the relative vapor pressure (RVP) of the air surrounding the material, such that $a_w = \text{RVP}/100$ when the material and the air surrounding the material are at equilibrium at a constant temperature [4,19].

The relationship between composition and the vapor pressure of dilute sugar solutions can be defined by Raoult’s law, which states that the relative lowering of the vapor pressure of the solvent is equal to the mole fraction of the solute [4,20]. Chirife et al. [21] found that $a_w$ values of solutions containing various sugars were lower than those predicted using Raoult’s law, even at $a_w$ values close to 0.97. Thus, several other equations have been suggested for predicting the $a_w$ of more concentrated sugar-containing solutions, as reviewed by Bell and Labuza [22] and Cazier and Gekas [23]. The water activities of sugar-containing solutions can also be calculated from the freezing point depression. Ferro Fontan and Chirife [24] found that such calculated $a_w$ values were quite similar to the $a_w$ values determined at 25°C. Water activity increases with increasing temperature if the water content of the material is kept constant. This temperature dependency can be modeled using the Clausius–Clapeyron equation [4].
2.4 MONO- AND DISACCHARIDES IN SOLID FORM

Mono- and disaccharides in solid form may be as stable crystals in the ordered, equilibrium state or as amorphous (noncrystalline) material in a metastable glassy state [4,6,12]. Formation of the glassy state results from the rapid solidification of food materials amorphous in structure with a random molecular order during various processes such as freezing, drying, extrusion, and rapid cooling [3,4]. Glassy solids are metastable supercooled liquids with an extremely high viscosity of about $10^{12}$ Pa·s [3,4,25], and they are capable of supporting their own weight [3]. In the glassy state, molecular mobility is low, restricted primarily to short-range rotational motions and vibrations involving only one to four chain atoms [26]. Thus, most chemical and structural changes occur extremely slowly in the glassy state.

2.4.1 CRYSTALLINE STATE AND MELTING OF SUGARS

Sperling [26] defined the crystalline state of a material as such a state in which material diffracts x-rays and exhibits first-order transition melting. Sugars crystallize into various crystal forms with a certain crystal structure that can be identified using x-ray diffraction (XRD) techniques. In the 1930s, Tuckey et al. [27] presented the XRD patterns for glucose, lactose, and sucrose. They stated that these common sugars could be differentiated from each other using x-rays because their different atomic arrangements produced distinct XRD patterns. XRD data for most sugars can be found, for example, from the PCPDFWIN powder diffraction database (version 1.30, 1997, by ICDD).

Sugars may crystallize as hydrates with different amounts of water. In addition, sugars existing in two or more anomeric forms, such as glucose and lactose, may crystallize into several different crystal forms; for example, glucose may crystallize into three crystal forms — $\alpha$-glucose monohydrate, anhydrous $\alpha$-glucose, and anhydrous $\beta$-glucose [1]. Different crystal forms may contain only one anomeric form, such as the crystal forms of glucose, or a mixture of two anomeric forms. Lactose, for example, may crystallize into at least seven different crystal forms, three of which contain mixtures of two anomeric forms. Figure 2.1 shows the intensities of the biggest peaks in the XRD patterns of six crystal forms of lactose as a function of diffraction angle. Some of those peaks can be used to identify the crystal forms of lactose — a peak at a diffraction angle of 10.5° for anhydrous $\beta$-lactose, at 16.4° for $\alpha$-lactose monohydrate, and at 22.1° for anhydrous crystals with $\alpha$- and $\beta$-lactose in a 5:3 molar ratio.

In addition to x-ray diffractometry, other techniques, mainly spectroscopic techniques, have been used in the identification of crystal forms. Earl and Parrish [31] found that five crystal forms of lactose could be identified from differences in their nuclear magnetic resonance (NMR) spectra. Vuataz [32]
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and Buckton et al. [33] found that \( \alpha \)-lactose monohydrate and \( \beta \)-lactose could be distinguished from each other by using near-infrared reflectance (NIR) spectra. Drapier-Beche et al. [34] found that the IR spectra of various crystal

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forms of lactose were quite similar, but slight differences, especially at wave numbers ranging from 500 to 1000 cm$^{-1}$, could be observed among the IR spectra of $\alpha$-lactose monohydrate, anhydrous $\alpha$-lactose, anhydrous $\beta$-lactose, and anhydrous crystals containing both $\alpha$- and $\beta$-lactose. The spectra of anhydrous crystals containing both $\alpha$- and $\beta$-lactose in a molar ratio of 5:3 and 3:2, however, were almost identical [34].

Different crystal forms of sugars with different crystal structures have different melting temperatures and melting enthalpies (Table 2.1); however, differences in the morphology of sugar crystals may be found even though their crystal form is the same. The morphology (or crystal shape) of sugar crystals depends not only on the crystal form of the sugar but also on the rate of crystal growth, temperature, and the presence of other compounds (even at low concentrations), as reviewed by Hartel [8]. For example, lactose crystals are tomahawk-shaped when grown slowly, but they are often needle shaped when grown at high supersaturation.

### 2.4.2 Water Sorption of Crystalline and Amorphous Sugars

The water sorption isotherm describes the relationship between the steady-state water content and water activity ($a_w$) of a material at constant temperature [19,39]. In water adsorption, material adsorbs water when stored at various relative humidities higher than the initial equilibrium relative humidity (ERH) of material (ERH = $a_w \cdot 100$) at the same temperature, resulting in the material gaining weight. In water desorption, a material desorbs water when stored at relative humidities lower than the initial ERH of material at the same temperature, resulting in a loss of weight of the material. Water sorption of dehydrated material is usually determined gravimetrically by weighing the samples stored at various relative humidities (established using various saturated salt solutions) at constant temperature and observing the changes in water content as a function of storage time [4,19].

Several water sorption isotherm models have been used in modeling the water sorption of food materials (i.e., steady-state water contents as a function of $a_w$), as reviewed by van den Berg and Bruin [39] and Iglesias and Chirife [40]. The most frequently used models for food materials are the Guggenheim–Anderson–de Boer (GAB) and Brunauer–Emmett–Teller (BET) models [4,22]. The biggest difference between these models is the range of water activity over which they are usually applicable: 0 to 0.5 for the BET model and a wide $a_w$ range for the GAB model [4,22]. Jouppila and Roos [41] tested the applicability of several water sorption isotherm models to modeling the water sorption of freeze-dried lactose and milk products. They found that most of the models tested could be used to predict the water sorption of freeze-dried milk products, but the GAB model was found to be the most applicable for such products.
<table>
<thead>
<tr>
<th>Material [Ref.]</th>
<th>( T_m ) (°C, onset value)</th>
<th>( T_m ) (°C, peak value)</th>
<th>( \Delta H_m ) (J g(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pentose-monosaccharides</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \alpha )-Arabinose [35]</td>
<td>150</td>
<td>160</td>
<td></td>
</tr>
<tr>
<td>( \alpha )-Ribose [35]</td>
<td>70</td>
<td>86</td>
<td></td>
</tr>
<tr>
<td>( \alpha )-Xylose [35]</td>
<td>143</td>
<td>157</td>
<td></td>
</tr>
<tr>
<td><strong>Hexose-monosaccharides</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \alpha )-Fucrose [35]</td>
<td>133</td>
<td>145</td>
<td></td>
</tr>
<tr>
<td>( \alpha )-Galactose [35]</td>
<td>163</td>
<td>170</td>
<td></td>
</tr>
<tr>
<td>( \alpha )-Glucose [35]</td>
<td>143</td>
<td>158</td>
<td></td>
</tr>
<tr>
<td>( \alpha )-Mannose [35]</td>
<td>120</td>
<td>134</td>
<td></td>
</tr>
<tr>
<td>( \alpha )-L-Rhamnose monohydrate [35]</td>
<td>86</td>
<td>99</td>
<td></td>
</tr>
<tr>
<td>( \alpha )-Fructose [35]</td>
<td>108</td>
<td>127</td>
<td></td>
</tr>
<tr>
<td>( \alpha )-Fructose [36]</td>
<td>113( ^c )</td>
<td>117</td>
<td>154</td>
</tr>
<tr>
<td>( \alpha )-L-Fucose [35]</td>
<td>133</td>
<td>145</td>
<td></td>
</tr>
<tr>
<td>( \alpha )-Lactose, anhydrous, stable [34]</td>
<td>216( ^b )</td>
<td>—</td>
<td>122</td>
</tr>
<tr>
<td>( \alpha )-Lactose, anhydrous, stable [37]</td>
<td>216( ^a )</td>
<td>—</td>
<td>121</td>
</tr>
<tr>
<td>( \alpha )-Lactose monohydrate [34]</td>
<td>215( ^b )</td>
<td>—</td>
<td>134</td>
</tr>
<tr>
<td>( \alpha )-Lactose monohydrate [35]</td>
<td>—</td>
<td>214</td>
<td></td>
</tr>
<tr>
<td>( \alpha )-Lactose monohydrate [37]</td>
<td>—</td>
<td>220</td>
<td></td>
</tr>
<tr>
<td>( \alpha )-Lactose monohydrate [38]</td>
<td>—</td>
<td>220</td>
<td></td>
</tr>
<tr>
<td>( \beta )-Lactose [34]</td>
<td>225( ^b )</td>
<td>—</td>
<td>198</td>
</tr>
<tr>
<td>( \beta )-Lactose [37]</td>
<td>235( ^a )</td>
<td>—</td>
<td>211</td>
</tr>
<tr>
<td>Lactose, anhydrous mixture of ( \alpha )- and ( \beta )-lactose in a molar ratio of 5:3 [34]</td>
<td>209( ^b )</td>
<td>—</td>
<td>125</td>
</tr>
<tr>
<td>Lactose, anhydrous mixture of ( \alpha )- and ( \beta )-lactose in a molar ratio of 3:2 [34]</td>
<td>219( ^b )</td>
<td>—</td>
<td>159</td>
</tr>
<tr>
<td>Maltose monohydrate [35]</td>
<td>104</td>
<td>123</td>
<td></td>
</tr>
<tr>
<td>( \alpha )-Melibiose, 0.5 mol H(_2)O/mol [35]</td>
<td>138</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Sucrose [35]</td>
<td>173</td>
<td>190</td>
<td></td>
</tr>
<tr>
<td>Sucrose [36]</td>
<td>184( ^c )</td>
<td>187</td>
<td>127</td>
</tr>
<tr>
<td>( \alpha ),( \alpha )-Trehalose dihydrate [35]</td>
<td>91</td>
<td>97</td>
<td></td>
</tr>
</tbody>
</table>

\( ^a \) The values were determined as the intersection of an extrapolation of the baseline with an extrapolation of the steepest portion of the fusion peak.

\( ^b \) The values were reported as melting points determined using DSC.

\( ^c \) The onset and peak temperatures were calculated automatically by software.
The water sorption isotherm for crystalline sugar is very different from the water sorption isotherm for amorphous sugar at the same temperature [4,22]. The water content of crystalline sugar is much smaller than that of amorphous sugar at low \( a_w \) values, but at high \( a_w \) values water begins to dissolve the crystal surface, resulting in a drastic increase in water sorption [22]. It has been suggested that the small water gain of crystalline sugar at low \( a_w \) values is due to water interaction occurring via hydrogen bonds only with the hydroxyl groups on the surface of the crystal [22]. Thus, a decrease in the particle size of crystalline sugar results in an increase in surface area and water content. The water sorption of crystalline and amorphous sugars has been determined in many studies (see, for example, Iglesias and Chirife [40]). More recent water sorption data have been reported for freeze-dried lactose [42,43], freeze-dried sucrose [42], freeze-dried trehalose [44], and crystalline sucrose with different particle sizes [45].

The water sorption behavior of materials is affected by temperature [19,22]. The water contents of materials decrease with increasing temperature at constant \( a_w \) values, and the \( a_w \) values of materials increase with increasing temperature at constant water content (see Section 2.3.3). The water sorption behavior of freeze-dried lactose has been studied at various temperatures (14, 24, and 34\(^\circ\)C) [46,47]. Temperature also affects the \( a_w \) values (= ERH/100) of saturated salt solutions, which is important to take into account when various relative humidities are established using such solutions, for example, in vacuum desiccators when determining the water sorption properties of materials. The effect of temperature on the \( a_w \) values of saturated salt solutions that are most often used in water sorption studies was modeled by Labuza et al. [48]. They found a linear relationship between \( \ln a_w \) and \( T^{-1} \) (degrees Kelvin) when \( a_w \) values were determined using saturated salt solutions stored at 25, 30, and 45\(^\circ\)C. They presented equations for each saturated salt solution which can be used in the calculation of \( a_w \) values at various temperatures.

In many studies, the occurrence and rate of the time-dependent crystallization of amorphous sugars have been determined gravimetrically from the loss of sorbed water (see Section 2.5.2.1.1); however, the packing of molecules affects the water sorption behavior of amorphous sugars. In dense glucose glass, the adsorption of water occurs mainly on only the surfaces of the glassy particles because of the absence of pores penetrable by water [49], whereas freeze-dried sugars are very porous, allowing quick water sorption throughout the material.

Water sorption behavior has also been used to determine the content of amorphous sugar in predominantly crystalline sugar. Even small amounts of amorphous sugar resulting, for example, from milling may alter the properties of sugar powder, causing variation and subsequent problems, especially in pharmaceutical processes [50]. Saleki-Gerhardt et al. [51] found a linear relationship between water content and the content of freeze-dried sucrose (0 to 100%) in crystalline sucrose when stored at relative humidities ranging from...
TABLE 2.2
Glass Transition Temperatures \( (T_g, \text{ Onset Value}) \) Determined Using DSC at Scanning Rate of 5°C/min and \( k \) Values for Various Anhydrous Amorphous Sugars and Products Containing Amorphous Sugars

<table>
<thead>
<tr>
<th>Material [Ref.]</th>
<th>( T_g ) (°C)</th>
<th>( k^a )</th>
<th>( k^b )</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pentose-monosaccharides</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ribose, melt [35]</td>
<td>–20</td>
<td>—</td>
<td>3.02</td>
</tr>
<tr>
<td>Xylose, melt [35]</td>
<td>6</td>
<td>—</td>
<td>3.79</td>
</tr>
<tr>
<td><strong>Hexose-monosaccharides</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fructose, melt [35]</td>
<td>5</td>
<td>—</td>
<td>3.76</td>
</tr>
<tr>
<td>Fucose, melt [35]</td>
<td>26</td>
<td>—</td>
<td>4.37</td>
</tr>
<tr>
<td>Galactose, melt [35]</td>
<td>30</td>
<td>—</td>
<td>4.49</td>
</tr>
<tr>
<td>Glucose, melt [53]</td>
<td>37c</td>
<td>—</td>
<td>4.69</td>
</tr>
<tr>
<td>Glucose, melt [35]</td>
<td>31</td>
<td>—</td>
<td>4.52</td>
</tr>
<tr>
<td>Mannose, melt [35]</td>
<td>25</td>
<td>—</td>
<td>4.34</td>
</tr>
<tr>
<td>Rhamnose, melt [35]</td>
<td>–7</td>
<td>—</td>
<td>3.40</td>
</tr>
<tr>
<td>Rhamnose, melt [54]</td>
<td>37c</td>
<td>—</td>
<td>4.69</td>
</tr>
<tr>
<td>Sorbose, melt [35]</td>
<td>19</td>
<td>—</td>
<td>4.17</td>
</tr>
<tr>
<td><strong>Disaccharides</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lactose, freeze-dried [42]</td>
<td>101</td>
<td>—</td>
<td>6.57</td>
</tr>
<tr>
<td>Lactose, freeze-dried [55]</td>
<td>97</td>
<td>6.7</td>
<td>6.45</td>
</tr>
<tr>
<td>Lactose, freeze-dried [56]</td>
<td>105</td>
<td>6.6</td>
<td>6.69</td>
</tr>
<tr>
<td>Lactose, spray-dried [57]</td>
<td>104c</td>
<td>3.8</td>
<td>6.66</td>
</tr>
<tr>
<td>Lactose, spray-dried [58]</td>
<td>101</td>
<td>6.2</td>
<td>6.57</td>
</tr>
<tr>
<td>Lactose, spray-dried [59]</td>
<td>105</td>
<td>6.9</td>
<td>6.69</td>
</tr>
<tr>
<td>Lactulose, melt [60]</td>
<td>79</td>
<td>—</td>
<td>5.92</td>
</tr>
<tr>
<td>Maltose, freeze-dried [61]</td>
<td>87</td>
<td>6</td>
<td>6.16</td>
</tr>
<tr>
<td>Maltose, melt [53]</td>
<td>91c</td>
<td>—</td>
<td>6.28</td>
</tr>
<tr>
<td>Melibiose, melt [35]</td>
<td>85</td>
<td>—</td>
<td>6.10</td>
</tr>
</tbody>
</table>

8 to 32% at 30°C. They concluded that determination of water sorption is a very sensitive technique for detecting amorphous solids at very low values (even 1%) in predominantly crystalline sugar. Buckton and Darcy [50] were able to distinguish lactose powder containing only 0.125% spray-dried lactose from lactose powder containing 100% \( \alpha \)-lactose monohydrate because of the differences in weight gain during storage at low relative humidities at 25°C.

### 2.4.3 Glass Transition and Plasticization of Amorphous Sugars

Glass transition is a second-order phase transition (i.e., state transition) that is characteristic for each amorphous material [4]. Glass transition takes place
over a particular temperature range, and it can be characterized by changes or discontinuities in certain thermodynamic and physical properties (heat capacity, thermal expansion, specific volume, and viscosity) occurring in the glass transition temperature range, as reviewed by Kauzmann [52], White and Cakebread [3], Sperling [26], and Roos [4]. The glass transitions of various food materials are most often determined using differential scanning calorimetry (DSC) to identify step changes in heat capacity [4]. The glass transition temperature ($T_g$) usually refers to the onset temperature [42] or midpoint temperature [53] of the glass transition temperature range. Glass transition temperatures of various anhydrous mono- and disaccharides determined using DSC are shown in Table 2.2. Glass transition temperatures of sugars are shown

### Table 2.2 (cont.)

<table>
<thead>
<tr>
<th>Material [Ref.]</th>
<th>$T_g$ (°C)</th>
<th>$k^a$</th>
<th>$k^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sucrose, freeze-dried [62]</td>
<td>62</td>
<td>4.7</td>
<td>5.43</td>
</tr>
<tr>
<td>Sucrose, freeze-dried [63]</td>
<td>74$^d$</td>
<td>6.7</td>
<td>5.78</td>
</tr>
<tr>
<td>Sucrose, spray-dried [57]</td>
<td>77$^c$</td>
<td>9.1</td>
<td>5.87</td>
</tr>
<tr>
<td>α,α-Trehalose, melt [35]</td>
<td>100</td>
<td>—</td>
<td>6.54</td>
</tr>
<tr>
<td>Trehalose, freeze-dried [64]</td>
<td>85</td>
<td>—</td>
<td>6.10</td>
</tr>
</tbody>
</table>

**Sugar-containing model systems**

<table>
<thead>
<tr>
<th>Material [Ref.]</th>
<th>$T_g$ (°C)</th>
<th>$k^a$</th>
<th>$k^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactose–albumin (3:1), spray-dried [59]</td>
<td>108</td>
<td>8.0</td>
<td>6.77</td>
</tr>
<tr>
<td>Lactose–gelatin (3:1), spray-dried [59]</td>
<td>113</td>
<td>7.9</td>
<td>6.92</td>
</tr>
<tr>
<td>Lactose–Na-caseinate (3:1), spray-dried [59]</td>
<td>104</td>
<td>7.2</td>
<td>6.66</td>
</tr>
<tr>
<td>Lactose–sucrose (1.8:1) with invertase, freeze-dried [65]</td>
<td>60</td>
<td>5.8</td>
<td>5.37</td>
</tr>
<tr>
<td>Lactose–sucrose–carrageenan (1.8:1:0.026) with invertase, freeze-dried [65]</td>
<td>63</td>
<td>6.3</td>
<td>5.46</td>
</tr>
</tbody>
</table>

**Sugar-containing products**

<table>
<thead>
<tr>
<th>Material [Ref.]</th>
<th>$T_g$ (°C)</th>
<th>$k^a$</th>
<th>$k^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Horseradish, freeze-dried [66]</td>
<td>58</td>
<td>5.3</td>
<td>5.31</td>
</tr>
<tr>
<td>Skim milk, freeze-dried [55]</td>
<td>92</td>
<td>5.7</td>
<td>6.31</td>
</tr>
<tr>
<td>Skim milk with hydrolyzed lactose, freeze-dried [55]</td>
<td>49</td>
<td>8.0</td>
<td>5.05</td>
</tr>
<tr>
<td>Strawberry, freeze-dried [66]</td>
<td>36</td>
<td>4.7</td>
<td>4.66</td>
</tr>
</tbody>
</table>

$^a$ The $k$ values were calculated using the Gordon–Taylor equation.

$^b$ The $k$ values were calculated from experimental $T_g$ values for anhydrous materials using Equation 2.2 [35].

$^c$ Scanning rate was 10°C min$^{-1}$. The midpoint temperature of glass transition was taken as $T_g$.

$^d$ Scanning rate was 10°C min$^{-1}$. It was not reported which temperature of glass transition was taken as $T_g$. © 2006 by Taylor & Francis Group, LLC
Carbohydrates in Food

to increase with increasing molecular weight [35], and the \( T_g \) values of mono-saccharides are much lower than those of disaccharides. A similar effect of molecular weight on \( T_g \) values has also been observed with higher molecular weight carbohydrates, such as maltooligosaccharides [53] and maltodextrins [61,67]. Table 2.2 illustrates the significant differences in glass transition temperatures of sugars with the same molecular weight. Such differences may be due to the molecular structure of sugars and interactions between sugar molecules.

Glass transition can also be determined from changes in molecular mobility and from relaxations [4]. Roozen and Hemminga [68] found an increase in rotational mobility of spin probes in sucrose–water mixtures using electron spin resonance (ESR) spectroscopy at the glass transition temperature range determined using DSC. Lloyd et al. [58] used NMR spin relaxation to determine the \( T_g \) of lactose at various water contents. The \( T_g \) values obtained were quite similar to the \( T_g \) (onset) values determined using DSC. Söderholm et al. [69] used Raman spectroscopy to determine \( T_g \) for anhydrous amorphous glucose. Their \( T_g \) of 35°C for glucose is similar to the \( T_g \) values shown in Table 2.2. Noel et al. [54] studied the dielectric relaxation behavior of anhydrous glassy sugars. They found that the \( \alpha \) relaxation temperature (maximum in a tan\( \delta \) peak) was typically 20°C above the \( T_g \) (midpoint) determined using DSC.

Amorphous mono- and disaccharides, like almost all biomaterials, can be plasticized by water [4,6], and an increase in water content causes a decrease in the glass transition temperature. Water plasticization can occur if a material is stored at relative humidities that lead to an increase in the water content of the material (i.e., increased water sorption). The hygroscopicity of lactose glass was discovered in the 1930s [70]. Also, glucose and sucrose glasses were found to be very hygroscopic, as reviewed by White and Cakebread [3].

Storage of amorphous food materials at temperatures above their glass transition temperatures results in thermal plasticization of material; the viscosity decreases dramatically, and the molecular mobility increases, allowing translational motion of low-molecular-weight compounds [4,6]. Thus, the physical state of the material changes rapidly (i.e., the material undergoes glass transition). Glassy material becomes rubbery or leathery, sometimes even a viscous, syrup-like liquid with obvious viscous flow. In thermal plasticization, viscosity decreases and molecular mobility increases with increasing temperature difference (\( T - T_g \)) between the storage temperature (\( T \)) and the glass transition temperature (\( T_g \)).

The glass transition temperature of a sugar mixture depends on the proportion of different sugars in the mixture. Lower molecular weight sugar has been shown to plasticize higher molecular weight sugar; for example, fructose plasticizes sucrose [71] and sucrose plasticizes lactose and trehalose [63]. Mono- and disaccharides can be used as plasticizers for higher molecular weight carbohydrates; for example, the glass transition temperatures of a
maltodextrin–sucrose mixture decrease with increasing amounts of sucrose [67]. Correspondingly, the glass transition temperatures of amorphous sugars increase with increasing amounts of high-molecular-weight carbohydrates; for example, the presence of maltodextrin increases the glass transition temperature of juice solids rich in monosaccharides and makes the production of spray-dried juice powder possible [4,6]. Similarly, Gabarra and Hartel [72] found that the $T_g$ of the amorphous mixture of sucrose and corn syrup saccharides increased with an increasing proportion of corn syrup saccharides.

Water plasticization of amorphous mono- and disaccharides can be modeled using various equations, such as the Gordon–Taylor, Couchman–Karasz, and Huang equations [4]. The most often used equation is the Gordon–Taylor equation [73]:

$$T_g = \frac{w_1 T_{g1} + k w_2 T_{g2}}{w_1 + k w_2} \quad (2.1)$$

where $T_g$ is the glass transition temperature of the mixture containing solids and water; $w_1$ and $w_2$ are weight fractions of solids and water, respectively; $T_{g1}$ and $T_{g2}$ are the $T_g$ values of solids and water, respectively; and $k$ is a constant.

Amorphous materials with low water contents are often obtained by storing the samples at various relative humidities, resulting in various water contents, and amorphous materials with high water contents are obtained by adding liquid water to the material. The $T_{g1}$ value is often determined experimentally, such as by DSC, and the $T_{g2}$ value is usually taken from the literature. A $T_g$ of $-135^\circ C$ [74] is most frequently used for amorphous water [35,55,59,61,62,75], but other values, such as a $T_g$ of $-138^\circ C$ [76], have also been used [53,77]. The Gordon–Taylor equation has been used successfully to model the plasticization effect of water on, for example, lactose [55,59], maltose [61], and sucrose [62]. The $k$ values obtained using the Gordon–Taylor equation are shown in Table 2.2.

Roos [35] found the linear relationship between the $k$ values and $T_g$ (onset value) of anhydrous sugars, as given in Equation 2.2:

$$k = 0.0293 \times T_g + 3.61 \quad (2.2)$$

The $k$ values calculated using Equation 2.2 (Table 2.2) are quite similar to the $k$ values calculated using the Gordon–Taylor equation. The difference between these $k$ values is less than 0.8 in most cases; however, the difference was almost 3 for $k$ values obtained for freeze-dried skim milk with hydrolyzed lactose.

The plasticization effect of lower molecular weight carbohydrates on higher molecular weight carbohydrates can also be modeled using the Gordon–Taylor equation. Roos and Karel [67] and Gabarra and Hartel [72] successfully used...
the Gordon–Taylor equation to model the plasticization effect of sucrose on various maltodextrins \((k = 3)\) and corn syrup saccharides \((k = 0.3)\), respectively.

Frozen solutions containing mono- or disaccharides also have an amorphous phase. The glass transition temperature of the unfrozen solution is dependent on the amount of ice formed during freezing \([62]\). The glass transition temperature increases with an increasing concentration of solutes in the unfrozen solution. In solutions with maximum ice formation, the glass transition temperature of the maximally freeze-concentrated solution \(T_g'\) and the onset temperature of ice melting within maximally freeze-concentrated solution \(T_m'\) can be determined. \(T_g'\) and \(T_m'\) values have been shown to be independent of the initial solids concentration of solution \([6]\). As shown in Table 2.3, \(T_g'\) and \(T_m'\) values increase with increasing molecular weight.

### TABLE 2.3
Glass Transition Temperatures \((T_g', \text{Onset}), \text{Concentration} \,(C_g'), \text{and Onset Temperature of Melting of Ice} \,(T_m') \text{ of Maximally Freeze-Concentrated Matrix for Various Sugars and Sugar-Containing Products Determined Using DSC}

<table>
<thead>
<tr>
<th>Material [Ref.]</th>
<th>(T_g') (^{°C})</th>
<th>(C_g') (%)</th>
<th>(T_m') (^{°C})</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pentose-monosaccharides</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arabinose [35]</td>
<td>−66</td>
<td>79.3</td>
<td>−53</td>
</tr>
<tr>
<td>Ribose [35]</td>
<td>−67</td>
<td>81.4</td>
<td>−53</td>
</tr>
<tr>
<td>Xylose [35]</td>
<td>−65</td>
<td>78.9</td>
<td>−53</td>
</tr>
<tr>
<td><strong>Hexose-monosaccharides</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fructose [35]</td>
<td>−57</td>
<td>82.5</td>
<td>−46</td>
</tr>
<tr>
<td>Fucose [35]</td>
<td>−62</td>
<td>78.4</td>
<td>−48</td>
</tr>
<tr>
<td>Galactose [35]</td>
<td>−56</td>
<td>80.5</td>
<td>−45</td>
</tr>
<tr>
<td>Glucose [35]</td>
<td>−57</td>
<td>80.0</td>
<td>−46</td>
</tr>
<tr>
<td>Mannose [35]</td>
<td>−58</td>
<td>80.1</td>
<td>−45</td>
</tr>
<tr>
<td>Rhamnose [35]</td>
<td>−60</td>
<td>82.8</td>
<td>−47</td>
</tr>
<tr>
<td>Sorbose [35]</td>
<td>−57</td>
<td>81.0</td>
<td>−44</td>
</tr>
<tr>
<td><strong>Disaccharides</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lactose [35]</td>
<td>−41</td>
<td>81.3</td>
<td>−30</td>
</tr>
<tr>
<td>Maltose [61]</td>
<td>−41</td>
<td>82.5</td>
<td>−31</td>
</tr>
<tr>
<td>Maltose [35]</td>
<td>−42</td>
<td>81.6</td>
<td>−32</td>
</tr>
<tr>
<td>Melibiose [35]</td>
<td>−42</td>
<td>81.7</td>
<td>−32</td>
</tr>
<tr>
<td>Sucrose [62]</td>
<td>−46</td>
<td>80</td>
<td>−34</td>
</tr>
<tr>
<td>Sucrose ((T_g' \text{ and } T_m' [62]; C_g' [35]))</td>
<td>−46</td>
<td>81.7</td>
<td>−34</td>
</tr>
<tr>
<td>α,α-­Trehalose [35]</td>
<td>−40</td>
<td>81.6</td>
<td>−30</td>
</tr>
<tr>
<td><strong>Sugar-containing products</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Freeze-dried skim milk [55]</td>
<td>−50</td>
<td>79.5</td>
<td>−32</td>
</tr>
<tr>
<td>Freeze-dried skim milk with hydrolyzed lactose [55]</td>
<td>−65</td>
<td>83.1</td>
<td>−40</td>
</tr>
</tbody>
</table>
concentration of solids in the maximally freeze-concentrated solution ($C_g'$), however, is typically close to 80% for various mono- and disaccharides [35]. Low $T_g'$ and $T_m'$ values of maximally freeze-concentrated solutions containing monosaccharides explain the difficulties often observed in the freeze-drying of monosaccharide-containing solutions. Also, storage of frozen products at temperatures higher than their $T_g'$ and $T_m'$ values may lead to defects in their quality. In this case, the unfrozen phase in the frozen product is partially freeze-concentrated and in a rubbery state, and various changes (e.g., recrystallization of ice resulting in bigger ice crystals) may occur in the frozen product.

### 2.4.4 Critical Values for Water Content and Storage Relative Humidity

Critical values for water content and storage relative humidity at a certain temperature have been defined as those values for water content and storage relative humidity, respectively, that depress the glass transition temperature of a material to that certain temperature [66]. Such critical values at room temperature are important to determine because many food and pharmaceutical products containing amorphous sugars are often stored at room temperature. Table 2.4 shows critical water contents and storage relative humidities for various mono- and disaccharides, as well as amorphous sugar containing materials at room temperature. If the storage relative humidity and the water content of a material are higher than these critical values, the glass transition temperature of the material decreases to below room temperature, and time-dependent changes (e.g., stickiness, caking, crystallization, and nonenzymatic browning) may occur at room temperature. Roos [66] showed that critical water content values increased with increasing molecular weight. Critical water content values for monosaccharides and products containing monosaccharides (freeze-dried skim milk with hydrolyzed lactose and strawberries) are much lower than for disaccharides and products containing disaccharides (Table 2.4).

The critical values for water content and storage relative humidity have also been predicted from changes in $\alpha$-lactose monohydrate content during storage [80] and changes in the water sorption behavior of amorphous sugar containing materials during storage at various relative humidities [79,81,82]. Changes in water sorption behavior occur because water-plasticized amorphous sugar may crystallize time dependently. This results in a lowered water content of the material because sugar crystals sorb much less water than amorphous sugars [22]. The critical values predicted from changes in $\alpha$-lactose monohydrate content and the water sorption behavior of dehydrated milk products were found to agree well with the values obtained using $T_g$ values (Table 2.4). These values are quite similar to the values published by Supplee [83], who reported in 1926 that the greatest instability in the sorption isotherm of milk powders occurred at relative humidities ranging from 40 to 50% at 25°C.
TABLE 2.4
Critical Values for Water Content and Storage Relative Humidity (RH) for Various Amorphous Sugars and Amorphous-Sugar-Containing Products

<table>
<thead>
<tr>
<th>Material [Ref.]</th>
<th>Temperature (°C)</th>
<th>Critical Water Content (g water/100 g solids)</th>
<th>Critical Storage RH (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Monosaccharides</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fucose [60]</td>
<td>25</td>
<td>0.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>—</td>
</tr>
<tr>
<td>Galactose [60]</td>
<td>25</td>
<td>0.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>—</td>
</tr>
<tr>
<td>Glucose [60]</td>
<td>25</td>
<td>0.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>—</td>
</tr>
<tr>
<td><strong>Disaccharides</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lactose [66]</td>
<td>25</td>
<td>7.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>33</td>
</tr>
<tr>
<td>Lactose, freeze-dried [55]</td>
<td>24</td>
<td>6.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>37</td>
</tr>
<tr>
<td>Lactose, spray-dried [59]</td>
<td>23</td>
<td>7.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>37</td>
</tr>
<tr>
<td>Lactulose [60]</td>
<td>25</td>
<td>5.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>—</td>
</tr>
<tr>
<td>Maltose [60]</td>
<td>25</td>
<td>6.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>—</td>
</tr>
<tr>
<td>Melibiose [60]</td>
<td>25</td>
<td>6.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>—</td>
</tr>
<tr>
<td>Sucrose [66]</td>
<td>25</td>
<td>4.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>23</td>
</tr>
<tr>
<td>Trehalose [60]</td>
<td>25</td>
<td>7.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>—</td>
</tr>
</tbody>
</table>

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### Sugar-containing model systems

<table>
<thead>
<tr>
<th>Product Description</th>
<th>Temperature</th>
<th>Texture Acceptance Limit</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactose–albumin (3:1), spray-dried [59]</td>
<td>23</td>
<td>6.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>35</td>
</tr>
<tr>
<td>Lactose–gelatin (3:1), spray-dried [59]</td>
<td>23</td>
<td>7.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>36</td>
</tr>
<tr>
<td>Lactose–Na-caseinate (3:1), spray-dried [59]</td>
<td>23</td>
<td>7.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>36</td>
</tr>
<tr>
<td>Lactose–whey protein isolate (WPI) (3:1), spray-dried [59]</td>
<td>23</td>
<td>6.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>36</td>
</tr>
</tbody>
</table>

### Sugar-containing products

<table>
<thead>
<tr>
<th>Product Description</th>
<th>Temperature</th>
<th>Texture Acceptance Limit</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Freeze-dried horseradish [66]</td>
<td>25</td>
<td>3.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>21</td>
</tr>
<tr>
<td>Freeze-dried skim milk [55]</td>
<td>24</td>
<td>7.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>37</td>
</tr>
<tr>
<td>Freeze-dried skim milk with hydrolyzed lactose [55]</td>
<td>24</td>
<td>2.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>16</td>
</tr>
<tr>
<td>Freeze-dried strawberries [66]</td>
<td>25</td>
<td>1.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7</td>
</tr>
<tr>
<td>Oven-dried apple slices [78]</td>
<td>20</td>
<td>3.5&lt;sup&gt;c&lt;/sup&gt;</td>
<td>18&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Skim milk powder [79]</td>
<td>20</td>
<td>8.4&lt;sup&gt;d&lt;/sup&gt;</td>
<td>43</td>
</tr>
<tr>
<td>Spray-dried skim milk [80]</td>
<td>20–25</td>
<td>7.5–8.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>—</td>
</tr>
<tr>
<td>Spray-dried whole milk [80]</td>
<td>20–25</td>
<td>6.5–7.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>—</td>
</tr>
<tr>
<td>Spray-dried skim milk [81]</td>
<td>25</td>
<td>6.4&lt;sup&gt;d&lt;/sup&gt;</td>
<td>40</td>
</tr>
<tr>
<td>Spray-dried ultrafiltration retentate skim milk [82]</td>
<td>20</td>
<td>8.5–9.5&lt;sup&gt;d&lt;/sup&gt;</td>
<td>50</td>
</tr>
</tbody>
</table>

<sup>a</sup> The values were based on determination of \( T_g \) values and calculated using the \( k \) values obtained using Equation 2.2 [35].

<sup>b</sup> The values were based on determination of \( T_g \) values and calculated using the \( k \) values obtained using the Gordon–Taylor equation.

<sup>c</sup> The values were based on the \( a_w \) value defined as texture acceptance limit (i.e., beginning of loss of crispness) with corresponding water content from water sorption data.

<sup>d</sup> The values were based on changes in water sorption behavior during storage.

<sup>e</sup> The values were based on changes in \( \alpha \)-lactose monohydrate content during storage.
2.5 CRYSTALLIZATION OF MONO- AND DISACCHARIDES

A classical description of crystallization usually includes three steps: (1) nucleation, (2) propagation or growth, and (3) maturation or recrystallization [4,25]. Hartel’s description of crystallization [8], however, includes four steps. An additional step — generation of a supersaturated state — is incorporated into the description of crystallization prior to the three classical steps mentioned above. Such an addition is very well founded because establishing a supersaturated solution or supercooled melt is a prerequisite for crystallization. In a supersaturated solution or in a supercooled melt, there is a thermodynamic driving force for crystallization; however, the thermodynamic driving force has to exceed the critical values of supersaturation (solution) and supercooling (melt) until crystallization occurs [12].

Nucleation, or the formation of nuclei (the incipient crystalline phase), is typically classified as primary or secondary nucleation [12]. The presence or absence of crystals of the crystallizing compound in the system prior to nucleation defines whether primary or secondary nucleation occurs. Primary nucleation occurs in systems that do not contain crystals of the crystallizing compound prior to nucleation, but secondary nucleation requires the presence of crystals of the crystallizing compound [12]. Primary nucleation can be classified into homogeneous and heterogeneous nucleation. Homogeneous nucleation is based on molecular accretion — the molecules form clusters of molecules that reach a stable size [7]. Heterogeneous nucleation is based on the presence of a foreign surface (dust particles or microscopic structures in the vessel wall) which acts as a nucleating site and enables molecular orientation [7]. Heterogeneous nucleation generally occurs at much lower levels of supersaturation and supercooling than homogeneous nucleation, which is assumed to occur rarely in real-life situations [8]; however, secondary nucleation (formation of nuclei due to the presence of existing crystals) may occur at lower levels of supersaturation and supercooling than heterogeneous primary nucleation [8].

Propagation or the growth of crystals involves a series of steps in which crystallizing molecules move to the crystal interface and orient themselves into the crystal lattice [4,8,12]: (1) mutarotation to correct the anomeric form, (2) diffusion of crystallizing molecules to the crystal interface, (3) removal of the hydration water, (4) counter-diffusion of noncrystallizing molecules from the crystal interface, (5) orientation of molecules at the crystal–liquid interface, (6) incorporation of molecules into the crystal lattice, and (7) removal of latent heat. The rate of crystallization may be controlled by any of these steps, the rate of which can be influenced by changing conditions during crystallization [8]. In maturation, it has been suggested that crystal perfection and slow crystal growth via Ostwald ripening occur [6,25]. In recrystallization (or ripening),
the crystalline structure likely reorganizes to a lower energy state, generally without a change in the crystalline phase volume [8]. Such a change involves redistribution of crystallizing molecules from small crystals to large crystals.

### 2.5.1 Crystallization in Solutions

Sugar crystallizing is an important unit operation in the sugar refining process. Crystallization in supersaturated sugar solutions is used to produce pure crystalline sugar that is generally stable during storage. In a supersaturated solution, crystals may be formed until the solution approaches its equilibrium condition [12]. Hartel and Shastry [12] reviewed various factors affecting the crystallization of sucrose, lactose, glucose, and fructose. Operating parameters that affect nucleation include, for example, the extent of supersaturation, temperature, agitation rate, and presence of other substances (impurities). The rate and extent of nucleation affecting the quality of the final product (shape and size distribution of crystals) can be influenced by controlling these operating parameters. Operating parameters that affect crystal growth include, for example, the extent of supersaturation, temperature, agitation rate, source of nuclei (including seeding), and presence of other substances (impurities). The most important factor in controlling crystal growth is the extent of supersaturation. According to Hartel [8], the proper number of initial nuclei or seeds must be used to ensure production of large crystals that can be efficiently separated from the solution in sugar refining.

### 2.5.2 Amorphous State and Crystallization

Amorphous sugars, which can be considered as supersaturated and supercooled materials, have a temperature- and concentration-dependent thermodynamic driving force toward a crystalline, equilibrium state [4,12]. When amorphous sugars or products containing amorphous sugars are stored at temperatures above their $T_g$, crystallization of the amorphous sugars may occur due to increased molecular mobility, which allows translational motions of sugar molecules [3,25,26]. The rate of crystallization of amorphous sugars has been shown to increase with an increasing temperature difference $T - T_g$ due to decreasing viscosity and increasing diffusion and translational mobility [4,6]; thus, the storage temperature and water content of amorphous sugar are the key parameters in predicting the tendency of amorphous sugar to crystallize during storage. This is why the effect of thermal and water plasticization on the occurrence, kinetics, and leveling-off of crystallization, as well as on crystal forms, has been studied widely using various techniques.

Crystallization of amorphous sugar may be delayed by the presence of polysaccharides or other macromolecules and other sugars or various anomeric forms of sugars [12]. Polysaccharides may impede the diffusion of sugar molecules and cause steric hindrance, which results in decreased nucleation.
and crystal growth in amorphous sugars [12]. Also, the presence of anomeric forms of a sugar may also delay or even inhibit crystallization, as would any other sugar [1,84].

2.5.2.1 Effect of Plasticization on Crystallization

Water and thermal plasticization affect the occurrence and kinetics of crystallization in amorphous sugars. Crystallization of amorphous sugar may occur when the relative humidity during storage is higher than the critical storage relative humidity at the storage temperature. Such critical storage relative humidities at room temperature are shown in Table 2.4. Sugar crystallization rates have been shown to increase with increasing storage relative humidity at a constant temperature [32,38,43,55,57,79,85–89] or with increasing storage temperature at a constant water content [42,90–93].

The crystallization of amorphous sugars is often detected gravimetrically or by using XRD, thermoanalytical, and spectroscopic techniques. Crystallization data vary greatly, which may be due to the various techniques used in crystallization studies as well as differences in sample preparation, such as the preparation of sugar solutions, dehydration methods, and storage conditions prior to and during the crystallization study. Differences in sample preparation may lead to varying $\alpha$ and $\beta$ ratios with mutarotating sugars and to varying amounts of crystal nuclei present in the amorphous sugar. Using Fourier transform infrared (FTIR) spectroscopy, Mathlouthi et al. [94] found that amorphous sucrose produced by quench-cooling was completely amorphous, but amorphous sucrose produced by freeze-drying was microcrystalline. The presence of crystal nuclei or microcrystals may promote crystallization under favorable conditions.

2.5.2.1.1 Gravimetric Studies

The crystallization of amorphous sugars is often observed by gravimetric studies because the occurrence of sugar crystallization results in the loss of sorbed water. In the early 1900s, Supplee [83] observed the gradual loss of sorbed water in skim milk powder during 8 weeks of storage at a relative humidity higher than 50% at 25°C. Also, Herrington [70] produced lactose glass (i.e., amorphous lactose) from lactose solution by rapid cooling and water evaporation and found that amorphous lactose quickly adsorbed water from the air until crystallization with a concomitant loss of sorbed water began. In many studies, the occurrence and the rate of crystallization of amorphous sugars have been related to the storage relative humidity (Table 2.5). These studies show that the rate of crystallization increases with increasing storage relative humidity. At high relative humidities, determination of the rate of crystallization from the loss of sorbed water may be difficult because the adsorption of water by amorphous material and the rapid crystallization resulting in the loss of sorbed water occur simultaneously.
Crystallization of lactose in dehydrated milk products or lactose–protein mixtures is also often observed from the loss of sorbed water [32,55,59, 79,81,96]. Crystallization of lactose in dehydrated milk products has been found to occur more slowly than crystallization in pure lactose [55,96]. Also, Haque and Roos [59] reported that the crystallization of lactose in lactose–protein mixtures occurred more slowly when stored at a relative humidity of 54% at 23°C than crystallization in pure lactose. At higher relative humidities (66% and 76%), however, the rate of lactose crystallization was the same in lactose–protein mixtures and in pure lactose [59].

Iglesias and Chirife [95] found that the crystallization of sucrose occurred more slowly in freeze-dried mixtures of sucrose and polysaccharides (carboxymethylcellulose, guar gum, or sodium alginate) than in pure freeze-dried sucrose at a relative humidity of 54% and 35°C. They suggested that the delay in crystallization of sucrose may be due to interactions between sucrose and polysaccharide molecules as well as the increased viscosity of matrix in the presence of polysaccharides. Also, the presence of other sugars has been shown to delay crystallization. Saleki-Gerhardt and Zografi [63] found that the induction time for sucrose crystallization observed gravimetrically increased with increasing amount of lactose, trehalose, or raffinose (trisaccharide) in the freeze-dried mixture. When the content of lactose, trehalose, or raffinose was higher than 10% in the mixture, no crystallization was observed in 2 weeks. Saleki-Gerhardt and Zografi [63] also reported that the loss of sorbed water occurred more rapidly in pure freeze-dried sucrose than in freeze-dried sucrose containing 1% lactose, trehalose, or raffinose at a relative humidity of 32% at 30°C.

2.5.2.1.2 XRD Studies

X-ray diffraction techniques are often used to confirm the amorphous state of material at the beginning of crystallization studies and to observe the extent of crystallization from increasing intensities and areas of peaks in XRD patterns as a function of storage time. Palmer et al. [86] studied the extent of crystallization in spray-dried sucrose stored at 24°C at relative humidities of 30% and 32% based on the intensities of peaks at the diffraction angle (2θ) of 24.8° in the recorded XRD patterns. The results obtained using the XRD technique were similar to the results they obtained gravimetrically (see Table 2.5). Jouppila et al. [75,88] studied the extent of lactose crystallization in freeze-dried skim milk and freeze-dried lactose based on the intensities of peaks at the diffraction angles (2θ) of 19°, 20°, and 22°. The intensity of peaks at the diffraction angles (2θ) of 19 and 20° can be used to investigate the overall crystallinity of lactose, because most crystal forms of lactose yield peaks at those diffraction angles (Figure 2.1), the intensities of which were also followed by Biliaderis et al. [77]. The intensity of peaks at the diffraction angle of 22° can be used to study the crystallization of lactose as anhydrous crystals with α- and β-lactose in a molar ratio of 5:3, which was found to be the predominant crystal form when lactose crystallized in freeze-dried skim milk [75]. Haque and Roos [89] studied
the extent of lactose crystallization using the intensities of peaks at the diffraction angle of 20.9° (in addition to 19° and 20°), representing the formation of anhydrous β-lactose during storage. The extent of crystallization in freeze-dried lactose and skim milk was observed to increase during storage at and above relative humidities of 44% and 54%, respectively, at 24°C [75,88]. Biliaderis et al. [77] observed that crystallization occurred at and above a relative humidity of 54% in freeze-dried lactose and mixtures of lactose and pullulan in the ratios of 3:1 and 2:1 and at and above relative humidities of 64% in a freeze-dried mixture of lactose and pullulan in the ratio of 1:1. All of these studies confirmed that crystallization occurs at higher relative humidities in the presence of other substances than for pure amorphous sugars.

### TABLE 2.5

Occurrence of Crystallization and Time to Complete Crystallization of Various Sugars as Observed from Loss of Sorbed Water During Storage at Various Temperatures and Relative Humidities

<table>
<thead>
<tr>
<th>Material [Ref.]</th>
<th>Temperature (°C)</th>
<th>Relative Humidity (%)</th>
<th>Occurrence of Crystallization or Time to Complete Crystallization</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose, rapidly cooled melt that was powdered at –25°C [85]</td>
<td>25</td>
<td>4.6</td>
<td>No crystallization</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>8.6</td>
<td>500 d</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>11.8</td>
<td>150 d</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>16.2</td>
<td>25 d</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>24.0</td>
<td>5 d</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>28.2</td>
<td>4 d</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>33.6</td>
<td>3 d</td>
</tr>
<tr>
<td>Sucrose, spray-dried [85]</td>
<td>25</td>
<td>4.6, 8.6, 11.8</td>
<td>No crystallization</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>16.2</td>
<td>&gt;850 d</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>24.0</td>
<td>&gt;400 d</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>28.2</td>
<td>50 d</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>33.6</td>
<td>&lt;2 d</td>
</tr>
<tr>
<td>Sucrose, spray-dried [86]</td>
<td>24</td>
<td>30.0</td>
<td>28 d</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>32.5</td>
<td>4 d</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>33.5</td>
<td>&lt;2 d</td>
</tr>
<tr>
<td>Sucrose, spray-dried [57]</td>
<td>21</td>
<td>22</td>
<td>No crystallization</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>33</td>
<td>140 hr (5.8 d)</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>57</td>
<td>35 hr (1.5 d)</td>
</tr>
<tr>
<td>Sucrose, freeze-dried [95]</td>
<td>35</td>
<td>54</td>
<td>25 hr</td>
</tr>
<tr>
<td>Sucrose, freeze-dried [63]</td>
<td>30</td>
<td>32.4</td>
<td>10 hr</td>
</tr>
<tr>
<td>Lactose, spray-dried [96]</td>
<td>25</td>
<td>33.44</td>
<td>No crystallization</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>55</td>
<td>25 hr</td>
</tr>
</tbody>
</table>
2.5.2.1.3 Thermoanalytical Studies

Thermoanalytical methods can be used to follow the crystallization of amorphous carbohydrates directly or indirectly. In direct measurement (isothermal or dynamic), the heat released during sugar crystallization can be recorded, as crystallization usually occurs relatively quickly during the measurement. The longest measurements reported (2 days) have been carried out using isothermal microcalorimetry. In indirect measurement, the heat consumed during the melting of crystals formed during storage is recorded. Crystallization studied by indirect measurements occurs relatively slowly (days or weeks) under various storage conditions, and the samples are measured after certain storage times. Indirect measurement is usually dynamic; that is, the sample is

---

TABLE 2.5 (cont.)
Occurrence of Crystallization and Time to Complete Crystallization of Various Sugars as Observed from Loss of Sorbed Water During Storage at Various Temperatures and Relative Humidities

<table>
<thead>
<tr>
<th>Material [Ref.]</th>
<th>Temperature (°C)</th>
<th>Relative Humidity (%)</th>
<th>Occurrence of Crystallization or Time to Complete Crystallization</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactose, spray-dried [97]</td>
<td>Room temperature</td>
<td>57</td>
<td>14 hr</td>
</tr>
<tr>
<td>Lactose, spray-dried [57]</td>
<td>21</td>
<td>33</td>
<td>No crystallization</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>57</td>
<td>13 hr</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>75</td>
<td>10 hr</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>84</td>
<td>8 hr</td>
</tr>
<tr>
<td>Lactose, spray-dried [98]</td>
<td>25</td>
<td>75</td>
<td>6 hr</td>
</tr>
<tr>
<td>Lactose, spray-dried [99]</td>
<td>25</td>
<td>75</td>
<td>2 hr</td>
</tr>
<tr>
<td>Lactose, spray-dried [100]</td>
<td>22-23</td>
<td>54.5</td>
<td>24 hr</td>
</tr>
<tr>
<td></td>
<td>22-23</td>
<td>65.6</td>
<td>21 hr</td>
</tr>
<tr>
<td></td>
<td>22-23</td>
<td>76.1</td>
<td>21 hr</td>
</tr>
<tr>
<td>Lactose, freeze-dried [96]</td>
<td>25</td>
<td>55</td>
<td>8 hr</td>
</tr>
<tr>
<td>Lactose, freeze-dried [101]</td>
<td>20</td>
<td>53</td>
<td>27 hr</td>
</tr>
<tr>
<td>Lactose, freeze-dried [122]</td>
<td>24</td>
<td>44</td>
<td>32 d</td>
</tr>
<tr>
<td></td>
<td>54</td>
<td>8 d</td>
<td></td>
</tr>
<tr>
<td></td>
<td>66</td>
<td>2 d</td>
<td></td>
</tr>
<tr>
<td></td>
<td>76</td>
<td>36 hr (1.5 d)</td>
<td></td>
</tr>
<tr>
<td>Lactose, freeze-dried [56]</td>
<td>22-23</td>
<td>54.5</td>
<td>48 hr</td>
</tr>
<tr>
<td></td>
<td>22-23</td>
<td>76.1</td>
<td>21 hr</td>
</tr>
<tr>
<td>Trehalose, spray-dried [102]</td>
<td>25</td>
<td>53</td>
<td>7 hr</td>
</tr>
<tr>
<td></td>
<td>75</td>
<td>~2 hr</td>
<td></td>
</tr>
</tbody>
</table>
heated over the melting range of crystals. Indirect measurement is mainly used
to determine crystallization in starch.

Differential scanning calorimetry is probably the most frequently used
thermoanalytical technique in crystallization studies. In isothermal crystalli-
zation studies, DSC samples are heated quickly from ambient temperature to
a crystallizing temperature, and heat flow data are collected until a crystalli-
zation exotherm is complete. The temperatures at which isothermal crystalli-
zation studies are carried out are usually quite high in order to obtain the
crystallization exotherm in a couple of hours — for example, up to 155°C for
anhydrous amorphous lactose [42,71] and up to 95°C for anhydrous amor-
phous sucrose [71]. Various points in DSC thermograms have been used as a
measure of the kinetics of crystallization. Determining the time at the peak of
the exotherm gives the isothermal crystallization time [42,71], and determining
the time at the onset of the exotherm gives the induction time for crystallization
[92,93,103,104]. Also, in studies of crystallization kinetics, the crystallization
enthalpy has been determined from the area of the DSC exotherm [105,106].

Roos and Karel [42,71] determined the isothermal crystallization times of
freeze-dried lactose and sucrose at varying temperatures and water contents
and found that the isothermal crystallization time of lactose and sucrose
decreased with increasing \( T - T_g \). A similar relationship between the induction
time for crystallization and \( T - T_g \) was observed by Shamblin et al. [103]. The
induction times for crystallization have also been found to decrease with
increasing temperatures (constant water content), as reported by Kedward et
al. [92], who studied crystallization in freeze-dried lactose and sucrose (con-
taining 3.2% and 0.94% water, respectively), and by Mazzobre et al. [104],
who studied crystallization in freeze-dried lactose and mixtures containing
lactose and trehalose in the ratio of 4:1 (stored at a relative humidity of 33%
 prior to crystallization studies). Kedward et al. [93] determined the induction
times for crystallization in freeze-dried lactose at three water contents and at
various temperatures. They found a similar relationship between induction
time and temperature with a constant water content. Also, they found that the
temperature at which crystallization occurred decreased with increasing water
content of the freeze-dried lactose.

Isothermal crystallization measurements have also been carried out using
isothermal microcalorimetry in which crystallization of amorphous sugars can
be studied under various relative humidity conditions by determining the heat
released during crystallization of amorphous sugars. Briggner et al. [87] and
Sebhatu et al. [38] found that the time to occurrence of the crystallization exotherm
decreased with the increasing relative humidity to which spray-dried
lactose was exposed at 25°C. Similar behavior was found for spray-dried
sucrose stored at relative humidities ranging from 20 to 80% at 25°C [107]
and for spray-dried trehalose stored at relative humidities of 53% and 75% at
25°C [102].
In dynamic measurements performed using DSC, instant crystallization temperatures \( T_{cr} \) and crystallization enthalpies \( \Delta H_{cr} \) are usually determined. Differences in these values are due to differences in sample preparation, the heating rates used, and the water content of the materials. \( T_{cr} \) values have been determined for various dehydrated sugars and sugar-containing materials that have been stored at various relative humidities: freeze-dried lactose [42,55,71, 77,106,108], spray-dried lactose [57,59], freeze-dried lactose–trehalose mixture [106], freeze-dried sucrose [42,63,71,108], spray-dried sucrose [57], freeze-dried milk products [55], and spray-dried lactose–protein mixtures [59]. In all these studies, the \( T_{cr} \) values were found to decrease with increasing water content.

Roos and Karel [42,71] suggested that the increase in water content caused about an equal decrease in \( T_g \) and \( T_{cr} \). A linear relationship between \( T_{cr} \) and water content was found for freeze-dried sucrose [42,63], a freeze-dried sucrose–fructose (7:1) mixture [71], spray-dried lactose and sucrose [57], and a spray-dried lactose–protein (3:1) mixture [59]. Jouppila and Roos [55], however, found that lactose crystallized in freeze-dried milk products at higher temperatures than in pure lactose and that \( T_{cr} \) values were fairly constant at water contents ranging from 4 to 9 g/100 g solids. They presumed that other milk solids, such as protein, delayed lactose crystallization at those water contents. Also, Haque and Roos [59] found that the presence of proteins delayed lactose crystallization in all mixtures containing water. The presence of other sugars or polymeric compounds in a freeze-dried mixture has been found to increase the \( T_{cr} \) values of freeze-dried sucrose [63,72,77,103] and freeze-dried lactose [106].

The crystallization enthalpy \( \Delta H_{cr} \) can be determined by using dynamic measurement to find out the ratio of amorphous to crystalline sugar in the sample. Such a ratio can also be determined by taking isothermal measurements at the temperatures at which crystallization occurs within a practical time frame. Roos and Karel [105] determined the crystallization enthalpy of remaining amorphous lactose at intervals using dynamic DSC scanning from samples in which crystallization had occurred either at a constant water content or at constant relative humidity at various temperatures, giving various \( T - T_g \) values. They found that crystallization occurred more rapidly in samples with a constant water content, the initial \( T - T_g \) values of which were about the same as those of samples stored at constant relative humidity. The reason for the more rapid crystallization at a constant water content was the release of sorbed water during crystallization which plasticized the remaining amorphous lactose, decreased the \( T_g \) values, and at the same time increased the \( T - T_g \) values.

Also, solution calorimetry has been used to monitor crystallization of amorphous sugar. Gao and Rytting [14] found that freeze-dried sucrose stored at a relative humidity of 32% at 25°C crystallized completely in 3 days, which is in agreement with the values shown in Table 2.5.
2.5.2.1.4 Other Techniques

Other techniques, such as spectroscopic techniques, have seldom been used to monitor the time-dependent crystallization of amorphous sugars. Vuataz [32] suggested the use of NIR spectroscopy for determining the degree of lactose crystallization based on changes in absorbance values at certain wavelengths. Lane and Buckton [99] studied the crystallization of spray-dried lactose at a relative humidity of 75% at 25°C using NIR spectroscopy. They suggested that the crystallization of lactose could be detected from increases in intensities at certain wavelengths in NIR spectra. They found that crystals of α-lactose monohydrate were formed during 2 hours of storage, which was in agreement with their gravimetric studies under similar crystallization conditions.

Ottenhof et al. [108] used FTIR spectroscopy to study phase transitions in freeze-dried lactose and sucrose at temperatures ranging from 30 to 200°C at intervals of 5°C. They found that crystallization temperatures obtained by FTIR spectroscopy were in satisfactory agreement with $T_{cr}$ values obtained with DSC. Jørgensen et al. [109] studied the crystallization of lactose at a relative humidity of 92% at room temperature using Raman spectroscopy. The Raman spectroscopic data obtained indicated water sorption and the collapse of amorphous lactose during first 4 hours of storage followed by rapidly progressing crystallization of lactose after 4 hours of storage (in 2 to 3 hours) with a concomitant loss of sorbed water.

2.5.2.2 Kinetics of Crystallization

Crystallization data, including crystallinity in amorphous sugars as a function of time, have often been modeled using the Avrami equation [110], but also by using first-order reaction kinetics [4]. The Avrami equation has usually been used to model sugar crystallization data obtained using DSC and XRD techniques [75,91,92]. First-order reaction kinetics was used to model crystallization data obtained using gravimetry [95]. The temperature dependence of the kinetics of sugar crystallization has been modeled using the Williams–Landel–Ferry (WLF) equation [111], with which the kinetics of crystallization can be related to the glass transition and $T – T_g$ [4,6].

The Avrami equation, given in Equation 2.3, suggests a sigmoidal curve of crystallinity when plotted against time; that is, crystallization begins quite slowly then propagates at a constant rate and finally slows down at the end of the crystallization process:

$$\theta = 1 - e^{-kt^n}$$

where $\theta$ is crystallinity, $k$ is a rate constant, $t$ is time, and $n$ is the Avrami exponent.
The Avrami equation can be written in the form of Equation 2.4 for fitting the equation to the intensities and areas of peaks in XRD patterns or melting enthalpies obtained in DSC determinations with time of crystallization:

$$1 - \Theta = \frac{I_f - I_t}{I_f - I_0} = e^{-kt^n}$$  \hspace{1cm} (2.4)

where $I_f$ is the leveling-off value of the peak intensity, peak area, or melting enthalpy at which crystallization ceased under each crystallization condition; $I_t$ is the peak intensity, peak area, or melting enthalpy at time $t$; and $I_0$ is the peak intensity or peak area of 0 counts or the melting enthalpy of 0 J g$^{-1}$ for a noncrystalline, amorphous material.

The constants of the Avrami equation ($k$, $n$) can be used to calculate the half-time ($t_{1/2}$) (Equation 2.5), which is defined as the time required to achieve 50% of the maximum extent of crystallinity under certain crystallization conditions:

$$t_{1/2} = n \sqrt{-\frac{\ln 0.5}{k}}$$ \hspace{1cm} (2.5)

The relationship between the increasing intensities of the peaks at various diffraction angles in XRD patterns and storage time has been modeled using the Avrami equation [75,77,88,89]. Table 2.6 shows the values of the constants in the Avrami equation as well as estimated half-times for lactose crystallization obtained in those studies. The half-times for crystallization in freeze- and spray-dried lactose obtained in different studies were quite similar. The rate of lactose crystallization, as detected from half-times, was found to increase as storage relative humidity increased. Half-times ranged from 2 to 4 hours at a relative humidity of 76% and from 13 to 27 hours at a relative humidity of 54%. In most cases, the half-times obtained from XRD data predicted quicker crystallization than was observed from gravimetric data [56,59,88,89]. At a relative humidity of 44% at 24°C (where $T - T_g$ was 11°C), crystallization in freeze-dried lactose was found to occur much more slowly; it took about 30 days to achieve the maximum extent of crystallization (Table 2.5).

Also, the crystallization kinetics data obtained using DSC were found to follow the Avrami equation [91,92,104,106]. Arvanitoyannis and Blanshard [91] and Mazzobre et al. [104,106] found that half-times for crystallization in freeze-dried lactose and sucrose as well as freeze-dried mixtures of lactose with sucrose and trehalose decreased with increasing storage temperature. Kedward et al. [92] modeled the crystallization kinetics of freeze-dried lactose and sucrose (containing 3.2% and 0.94% water, respectively) during isothermal DSC scans at various temperatures using the Avrami equation. The relationship between the rate of crystallization (= half-time$^{-1}$) and temperature resembles
<table>
<thead>
<tr>
<th>Material [Ref.]</th>
<th>RH (%)</th>
<th>T (°C)</th>
<th>T – Tg (°C)</th>
<th>k (hr⁻¹)</th>
<th>n</th>
<th>Half-Time (hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water-plasticized amorphous lactose</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Freeze-dried lactose [88]</td>
<td>54</td>
<td>24</td>
<td>30</td>
<td>5.1×10⁻⁴</td>
<td>2.2</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>66</td>
<td>24</td>
<td>63</td>
<td>4.4×10⁻²</td>
<td>2.3</td>
<td>63</td>
</tr>
<tr>
<td></td>
<td>76</td>
<td>24</td>
<td>105</td>
<td>3.7×10⁻¹</td>
<td>0.5</td>
<td>105</td>
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<tr>
<td>Freeze-dried lactose [77]</td>
<td>54</td>
<td>25</td>
<td>7</td>
<td>9.3×10⁻⁴</td>
<td>3.4</td>
<td>13.2</td>
</tr>
<tr>
<td></td>
<td>64</td>
<td>25</td>
<td>50</td>
<td>0.8×10⁻³</td>
<td>1.8</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>75</td>
<td>25</td>
<td>—</td>
<td>1.1×10⁻⁴</td>
<td>4.0</td>
<td>1.1</td>
</tr>
<tr>
<td></td>
<td>84</td>
<td>25</td>
<td>—</td>
<td>6.2×10⁻¹</td>
<td>1.1</td>
<td>1.5</td>
</tr>
<tr>
<td>Freeze-dried lactose [89]</td>
<td>54</td>
<td>22–23</td>
<td>34</td>
<td>1.3×10⁻⁴</td>
<td>2.8</td>
<td>21.4</td>
</tr>
<tr>
<td></td>
<td>66</td>
<td>22–23</td>
<td>71</td>
<td>4.4×10⁻⁴</td>
<td>0.53</td>
<td>2.3</td>
</tr>
<tr>
<td></td>
<td>76</td>
<td>22–23</td>
<td>128</td>
<td>5.6×10⁻¹</td>
<td>0.46</td>
<td>1.6</td>
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</table>
### Water-plasticized model systems containing amorphous lactose

<table>
<thead>
<tr>
<th></th>
<th>a</th>
<th>b</th>
<th>c</th>
<th>d</th>
<th>e</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spray-dried lactose [89]</td>
<td>54</td>
<td>22–23</td>
<td>39</td>
<td>$1.1 \times 10^{-4}$</td>
<td>2.9</td>
</tr>
<tr>
<td></td>
<td>66</td>
<td>22–23</td>
<td>73</td>
<td>$4.9 \times 10^{-4}$</td>
<td>0.4</td>
</tr>
<tr>
<td></td>
<td>76</td>
<td>22–23</td>
<td>120</td>
<td>$5.1 \times 10^{-4}$</td>
<td>0.45</td>
</tr>
</tbody>
</table>

**Freeze-dried lactose–pullulan (3:1) [77]**

<table>
<thead>
<tr>
<th></th>
<th>a</th>
<th>b</th>
<th>c</th>
<th>d</th>
<th>e</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>54</td>
<td>25</td>
<td>2</td>
<td>$0.9 \times 10^{-4}$</td>
<td>3.2</td>
</tr>
<tr>
<td></td>
<td>64</td>
<td>25</td>
<td>37</td>
<td>$2.5 \times 10^{-4}$</td>
<td>4.1</td>
</tr>
<tr>
<td></td>
<td>75</td>
<td>25</td>
<td>—</td>
<td>$0.7 \times 10^{-3}$</td>
<td>2.7</td>
</tr>
<tr>
<td></td>
<td>84</td>
<td>25</td>
<td>—</td>
<td>$1.9 \times 10^{-4}$</td>
<td>1.3</td>
</tr>
</tbody>
</table>

**Freeze-dried lactose–pullulan (2:1) [77]**

<table>
<thead>
<tr>
<th></th>
<th>a</th>
<th>b</th>
<th>c</th>
<th>d</th>
<th>e</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>54</td>
<td>25</td>
<td>—2</td>
<td>$1.0 \times 10^{-11}$</td>
<td>2.9</td>
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<tr>
<td></td>
<td>64</td>
<td>25</td>
<td>38</td>
<td>$1.6 \times 10^{-8}$</td>
<td>3.6</td>
</tr>
<tr>
<td></td>
<td>75</td>
<td>25</td>
<td>—</td>
<td>$1.1 \times 10^{-3}$</td>
<td>2.3</td>
</tr>
<tr>
<td></td>
<td>84</td>
<td>25</td>
<td>—</td>
<td>$1.7 \times 10^{-4}$</td>
<td>2.4</td>
</tr>
</tbody>
</table>

**Freeze-dried lactose–pullulan (1:1) [77]**

<table>
<thead>
<tr>
<th></th>
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<th>b</th>
<th>c</th>
<th>d</th>
<th>e</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>64</td>
<td>25</td>
<td>23</td>
<td>$1.1 \times 10^{-9}$</td>
<td>4.2</td>
</tr>
<tr>
<td></td>
<td>75</td>
<td>25</td>
<td>—</td>
<td>$3.5 \times 10^{-4}$</td>
<td>3.9</td>
</tr>
<tr>
<td></td>
<td>84</td>
<td>25</td>
<td>—</td>
<td>$7.2 \times 10^{-6}$</td>
<td>4.6</td>
</tr>
</tbody>
</table>

### Water-plasticized milk products containing amorphous lactose

<table>
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<tr>
<th></th>
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<th>b</th>
<th>c</th>
<th>d</th>
<th>e</th>
</tr>
</thead>
<tbody>
<tr>
<td>Freeze-dried skim milk [75]</td>
<td>66</td>
<td>24</td>
<td>52</td>
<td>$1.5 \times 10^{-6}$</td>
<td>3.5</td>
</tr>
<tr>
<td></td>
<td>76</td>
<td>24</td>
<td>80</td>
<td>$1.2 \times 10^{-3}$</td>
<td>2.1</td>
</tr>
<tr>
<td></td>
<td>86</td>
<td>24</td>
<td>118</td>
<td>$1.7 \times 10^{-3}$</td>
<td>2.9</td>
</tr>
</tbody>
</table>

---

* Time to 50% of the leveling-off extent of crystallinity.
* a Extents of lactose crystallization as a function of storage time were observed from increasing intensities of peaks in XRD patterns at a diffraction angle of 2θ [28].
* b $T - T_g$ values were calculated using the estimated $T_g$ (onset) values obtained using the Gordon–Taylor equation [55]. Water contents used in the Gordon–Taylor equation were the predicted water contents obtained using the GAB model [43].
* c $T - T_g$ values were calculated using the experimental $T_g$ (midpoint) values determined using DSC.
* d $T - T_g$ values were obtained from M.K. Haque and Y.H. Roos (pers. comm., 2005).

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a normal distribution in shape (Figure 2.2), as suggested by Slade and Levine [6]. At temperatures below the glass transition temperature, molecular mobility is very low, thus the rate of crystallization is almost zero [26]. It has been suggested that the maximum rate of crystallization occurs between the glass transition and equilibrium melting temperatures, because nucleation occurs rapidly at temperatures above the glass transition temperature, and crystal growth occurs at temperatures close to the equilibrium melting temperature [6]. Kedward et al. [92] suggested that the maximum rate of lactose and sucrose crystallization occurs at 155°C and 135°C, with corresponding \( T - T_g \) values of 100°C and 70°C, respectively. A similar trend was also observed by Jouppila et al. [88] for the crystallization of freeze-dried lactose at various relative humidities at 24°C, detected using XRD, as shown in Figure 2.2. All the other half-times, shown in Table 2.6, increased with increasing relative humidities.

First-order reaction kinetics follow Equation 2.6 [4] and demonstrate a linear relationship between \( \ln \left( \frac{C}{C_0} \right) \) and time, the slope of which provides the first-order rate constant:

\[
-ln \left( \frac{C}{C_0} \right) = k_1 t
\]  

(2.6)

where \( C \) is the concentration at time \( t \), \( C_0 \) is the initial concentration, and \( k_1 \) is the first-order rate constant.
Iglesias and Chirife [95] used a similar relationship to model crystallization data obtained gravimetrically. The modeling of crystallization kinetics observed gravimetrically is infrequently reported in the literature. Iglesias and Chirife [95] found a linear relationship between the logarithm of the nondimensional water content \( \frac{X}{X_{\text{max}}} \), where \( X \) is the water content at time \( t \) and \( X_{\text{max}} \) is the maximum water content observed) and time after the maximum water content \( X_{\text{max}} \) was achieved (i.e., after an initial induction time). They reported a rate constant of \( 1.1 \times 10^{-1} \text{ hr}^{-1} \) for the crystallization of freeze-dried sucrose and rate constants ranging from \( 5.7 \times 10^{-4} \) to \( 6.3 \times 10^{-2} \text{ hr}^{-1} \) for the crystallization of sucrose in the presence of various polysaccharides at a relative humidity of 54% and 35°C.

It has been suggested that the WLF equation, given in Equation 2.7, successfully predicts the temperature dependence of mechanical and dielectric properties in amorphous materials over a temperature range from \( T_g \) to \( (T_g + 100{\text{°C}}) \) [111]:

\[
\log a_T = \frac{-C_1(T - T_g)}{C_2 + (T - T_g)}
\]

(2.7)

where \( a_T \) is the ratio of any mechanical or electrical relaxation time at temperature \( T \) to its value at a reference temperature, \( T_g \); \( C_1 \) and \( C_2 \) are constants.

The WLF model has been used to model the kinetics of freeze-dried lactose [42,71,105,106], a freeze-dried lactose–trehalose mixture [106], and freeze-dried sucrose [71,103] using the universal constants of 17.44 for \( C_1 \) and 51.6 for \( C_2 \) [111], suggesting an increasing rate of crystallization with increasing \( T - T_g \) from 0 to 100°C. The WLF model, however, suggests a leveling-off of the rate of crystallization at high \( T - T_g \) values. Peleg [112] suggested the use of calculated values for the constants \( C_1 \) and \( C_2 \) to reliably model the crystallization kinetics of amorphous sugars. He reported slightly lower values for \( C_1 \) (10.5 for amorphous lactose and 11.9 for amorphous sucrose) and higher values for \( C_2 \) (85.6 for amorphous lactose and 75.6 for amorphous sucrose).

Peleg [112] stated that crystallization experiments should be carried out within a wide temperature range, especially if there is variation in the data obtained in crystallization experiments, in order to reveal the curvature of the relationship between \( \log a_T \) and \( T - T_g \).

Biliaderis et al. [77] found that the WLF model fitted quite well to the crystallization data of freeze-dried lactose and mixtures of lactose and pullulan when the values of –8.95 and 10.6 were used for the constants \( C_1 \) and \( C_2 \), respectively. Mazzobre et al. [106] reported values of 3.09 and 50 for the constants \( C_1 \) and \( C_2 \), respectively, which were obtained from the crystallization data of freeze-dried lactose. The values of 5.78 and 50 for the constants \( C_1 \) and \( C_2 \), respectively, were obtained from crystallization data for a freeze-dried lactose–trehalose mixture [106]. Mazzobre et al. [106] concluded that the WLF
model fitted quite well to crystallization data obtained, but extrapolations beyond the temperature ranges used in the experiments may give misleading results.

### 2.5.2.3 Leveling-Off Extent of Crystallization

It has been shown in many studies that crystallization in amorphous lactose detected by various methods tends to level off to a certain value that can be defined as the leveling-off extent of lactose crystallization under particular crystallization conditions. The leveling-off extent of crystallization has been shown to depend on relative humidity at constant temperature [38,75,77, 87–89]. Jouppila et al. [75] found a parabolic relationship between the leveling-off extent of lactose crystallization in freeze-dried skim milk detected using an XRD technique at 24°C, with a maximum occurring at a relative humidity of 70%. Similar parabolic relationships can been found for dehydrated lactose and lactose-containing mixtures, as shown in Table 2.7. The relative humidities at which the maximum extent of crystallization was predicted to occur varied from 59 to 77%. The most divergent values were obtained from crystallization enthalpy data determined by isothermal microcalorimetry; however, differences in crystallization enthalpies may be found due to crystallization of lactose into various crystal forms under various crystallization conditions. Various crystal forms may also cause differences in leveling-off values determined using XRD; for example, the presence of anhydrous β-lactose, which yields no peak at the diffraction angle of 20° (which is generally used to evaluate the overall crystallinity of lactose), may result in lower leveling-off values.

Darcy and Buckton [113] found no differences in the crystallization enthalpies of spray-dried lactose stored at various relative humidities at 25°C when studied using isothermal microcalorimetry; however, they found that the crystallization enthalpy increased with increasing temperature from 25 to 60°C, as the relative humidity of the saturated salt solution decreased slightly with the increasing temperature. Also, the heat of crystallization was found to increase with decreasing relative humidity from 75 to 30% at a constant temperature. The authors suggested that differences in water sorption behavior due to differences in temperature and relative humidity affect the heat of crystallization, including the heat of sorption. Also, lactose was found to crystallize into various crystal forms under various crystallization conditions [113].

The leveling-off extent of lactose crystallization in freeze-dried skim milk was also related to water content as well as the temperature difference between the storage temperature and the glass transition temperature \((T - T_g)\) [75]. The maximum extent of lactose crystallization was predicted to be attained at a water content of 17% (w/w) or 20.5 g/100 g of solids (at a relative humidity of 70% at 24°C; calculated with the GAB model) and at a \(T - T_g\) value of
61°C, corresponding to the water content of 17% (w/w) [75]. Jouppila et al. [75] suggested that the reason for a low extent of lactose crystallization at low relative humidities, water contents, and $T - T_g$ values may be low molecular mobility and restricted diffusion of lactose molecules within the nonfat milk solids, as well as the effect of the crystals formed at the beginning of storage on further crystallization (hindrance or retardation). The reason for the low extent of lactose crystallization at high relative humidities, water contents, and $T - T_g$ values may be solubilization of lactose in the sorbed water and formation of a supersaturated lactose solution.

Various techniques, especially in pharmaceutical studies, have been used to detect the content of amorphous sugar in predominantly crystalline sugar. The lowest detection limit of 0.125% spray-dried lactose has been reported

<table>
<thead>
<tr>
<th>Sample [Ref.]</th>
<th>RH, T</th>
<th>$R^2$</th>
<th>Method Used To Study Lactose Crystallization</th>
</tr>
</thead>
<tbody>
<tr>
<td>Freeze-dried skim milk [75]</td>
<td>71%, 24°C</td>
<td>0.998</td>
<td>XRD; intensity of the peak at diffraction angle (2θ) of 19°</td>
</tr>
<tr>
<td>Freeze-dried skim milk [75]</td>
<td>70%, 24°C</td>
<td>0.998</td>
<td>XRD; intensity of the peak at diffraction angle (2θ) of 20°</td>
</tr>
<tr>
<td>Freeze-dried skim milk [75]</td>
<td>72%, 24°C</td>
<td>0.991</td>
<td>XRD; intensity of the peak at diffraction angle (2θ) of 22°</td>
</tr>
<tr>
<td>Freeze-dried lactose [88]</td>
<td>71%, 24°C</td>
<td>0.783</td>
<td>XRD; intensity of the peak at diffraction angle (2θ) of 20°</td>
</tr>
<tr>
<td>Freeze-dried lactose [77]</td>
<td>63%, 25°C</td>
<td>0.994</td>
<td>XRD; intensity of the peak at diffraction angle (2θ) of 19°</td>
</tr>
<tr>
<td>Freeze-dried mixture of lactose and pullulan at ratio of 3:1 [77]</td>
<td>70%, 25°C</td>
<td>0.904</td>
<td>XRD; intensity of the peak at diffraction angle (2θ) of 20°</td>
</tr>
<tr>
<td>Freeze-dried mixture of lactose and pullulan at ratio of 2:1 [77]</td>
<td>65%, 25°C</td>
<td>0.671</td>
<td>XRD; intensity of the peak at diffraction angle (2θ) of 19°</td>
</tr>
<tr>
<td>Freeze-dried mixture of lactose and pullulan at ratio of 2:1 [77]</td>
<td>67%, 25°C</td>
<td>0.463</td>
<td>XRD; intensity of the peak at diffraction angle (2θ) of 20°</td>
</tr>
<tr>
<td>Spray-dried lactose [87]</td>
<td>59%, 25°C</td>
<td>0.683</td>
<td>Isothermal microcalorimetry; crystallization enthalpy</td>
</tr>
<tr>
<td>Spray-dried lactose [38]</td>
<td>77%, 25°C</td>
<td>0.827</td>
<td>Isothermal microcalorimetry; crystallization enthalpy</td>
</tr>
</tbody>
</table>

* The relationship between the leveling-off extent of lactose crystallization and relative humidity was modeled using a second-order polynomial; the coefficients of determination ($R^2$) and methods used in obtaining crystallization data are shown.
Carbohydrates in Food

for water sorption measurements [50]. Low detection limits ranging from 0.5 to 2% have also been reported for isothermal microcalorimetry. Crystallization enthalpy has been shown to have a linear relationship with the content of spray-dried lactose in a mixture with α-lactose monohydrate (detection limits 2% [38], 1% [115], and 0.5% [114]) and with the content of spray-dried trehalose in a mixture with α-lactose monohydrate (detection limit 1% [102]). A detection limit of 5% was reported for DSC when the crystallization enthalpy of freeze-dried sucrose was determined at a heating rate of 10°C min⁻¹ from the mixture containing amorphous and crystalline sucrose [51]. Detection limits ranging from 5 to 10% have been reported for traditional XRD techniques. A linear relationship between crystallinity and the content of amorphous sugar in a mixture with crystalline sugar has been found for a mixture containing freeze-dried sucrose and crystalline sucrose (detection limit 10% [51]) and a mixture containing spray-dried lactose and α-lactose monohydrate (detection limit 5% [116]). Chen et al. [117], however, reported a detection limit of 0.37% for a mixture containing α-lactose monohydrate and freeze-dried lactose when crystallinity was detected using parallel-beam XRD and whole-pattern fitting was used to analyze diffraction patterns. Other methods that have been shown to be applicable for the determination of amorphous sugar content in predominantly crystalline mixtures include solid-state NMR (detection limit of 0.5% for spray-dried lactose [114]) and NIR spectroscopy (detection limit of 5% [116]).

2.5.2.4 Crystal Forms

Several amorphous sugars may crystallize into different crystal forms which can be identified by, for example, XRD and spectroscopic techniques. Storage conditions (relative humidity, temperature, and $T - T_g$) and the presence of other compounds during crystallization have been shown to affect into which crystal forms the sugar crystallizes. The crystallization of lactose into various crystal forms when stored at various storage conditions has been studied widely [38,75,87–89,96,101].

The various crystal forms of lactose detected by XRD patterns recorded from lactose and lactose-containing materials are shown in Table 2.8. In most studies, both freeze- and spray-dried lactose have been found to crystallize primarily as a mixture of α-lactose monohydrate and anhydrous β-lactose [38,87,96,101]. Buckton et al. [33] studied the crystallization of spray-dried lactose at a relative humidity of 75% at 20°C using NIR spectroscopy. They found that spray-dried lactose crystallized initially as anhydrous β-lactose, but during further storage the crystal form changed to α-lactose monohydrate. Haque and Roos [89] reported that both freeze- and spray-dried lactose crystallized as a mixture of α-lactose monohydrate, anhydrous β-lactose, and anhydrous crystals of α- and β-lactose in molar ratios of 5:3 and 4:1, respectively, when stored at relative humidities ranging from 54 to 76% at room
temperature. They also found that the proportion of anhydrous β-lactose decreased and the proportion of α-lactose monohydrate increased with increasing storage relative humidity in samples stored for 144 hours and as a function of storage time in samples stored at a relative humidity of 76% at room temperature.

The presence of other compounds in lactose-containing materials, such as other carbohydrates and milk solids (e.g., protein, salts, fat), probably affects crystallization behavior and the crystal forms of lactose produced during storage [75,96]. As shown in Table 2.8, it has been reported that lactose crystallizes into different crystal forms in freeze- and spray-dried milk — mainly as anhydrous crystals with α- and β-lactose in a molar ratio of 5:3 and as α-lactose monohydrate, respectively. Saito [119] and Drapier-Beche et al. [101] found that lactose in spray-dried milk also crystallizes as anhydrous β-lactose when stored at relative humidities up to 53%.

Differences in the crystal forms of lactose produced during storage may be due to thermal history and the concentration of lactose-containing solution prior to and during dehydration, because the equilibrium ratio of anomeric forms of lactose is shown to be affected by temperature, concentration, pH, and the presence of other compounds [8,12]. For example, the cooling and freezing rate prior to freeze-drying may affect the ratio of anomeric forms of lactose due to slow mutarotation at low temperatures. A different ratio of anomeric forms of lactose is obtained in lactose-containing solutions prepared for spray-drying because of the higher temperature at which mutarotation occurs rapidly. Mutarotation of lactose has also been found to occur in an amorphous state at a rate that depends on water content and temperature [121]. Roetman and van Schaik [121] found that mutarotation occurs under favorable conditions until a β/α ratio of about 1.25 is attained. That β/α ratio is considered to be a “universal” equilibrium for amorphous lactose.

In freeze- and spray-drying, a high concentration of lactose prior to and during drying may cause supersaturation of lactose and, probably, formation of nuclei, which may affect the crystallization behavior of lactose. Also, poor freezing and freeze-drying conditions (slow freezing, too high storage temperature of frozen solutions prior to freeze-drying, too low of a vacuum) may cause formation of crystal nuclei. The early nucleation process may affect the crystallization behavior of lactose (e.g., the crystal form into which it crystallizes [60,88]).

Identification of the crystal forms of sugars based on XRD patterns may sometimes be difficult because the peaks in XRD patterns may be fairly broad, probably because of the presence of small crystals, resulting in the overlap of some peaks. Evaluation of the amounts of various crystal forms is even more difficult. Figure 2.3 shows the determination of the crystal form of a lactose sample stored at a relative humidity of 44% at 24°C for 25 days. The three main crystal forms present in this sample are α-lactose monohydrate, anhydrous
<table>
<thead>
<tr>
<th>Material [Ref.]</th>
<th>T (°C)</th>
<th>RH (%)</th>
<th>Crystal Forms of Lactose Present</th>
</tr>
</thead>
<tbody>
<tr>
<td>Freeze-dried lactose [96]</td>
<td>25</td>
<td>55</td>
<td>Mixture of α-lactose monohydrate and anhydrous β-lactose</td>
</tr>
<tr>
<td>Freeze-dried lactose [101]</td>
<td>20</td>
<td>53</td>
<td>Mixture of α-lactose monohydrate and anhydrous β-lactose</td>
</tr>
<tr>
<td>Freeze-dried lactose [60,88]</td>
<td>24</td>
<td>44</td>
<td>Mixture of α-lactose monohydrate and anhydrous crystals of α- and β-lactose in a molar ratio of 5:3 (mainly), anhydrous β-lactose (traces), anhydrous crystals of α- and β-lactose in a molar ratio of 4:1 (traces)</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>54, 66, 76</td>
<td>Mixture of α-lactose monohydrate and anhydrous crystals of α- and β-lactose in a molar ratio of 5:3 (mainly), anhydrous crystals of α- and β-lactose in a molar ratio of 4:1 (traces), unstable α-lactose (traces)</td>
</tr>
<tr>
<td>Freeze-dried lactose [77]</td>
<td>25</td>
<td>54, 64, 75, 84</td>
<td>Mixture of anhydrous β-lactose, α-lactose monohydrate, and anhydrous crystals of α- and β-lactose in a molar ratio of 5:3</td>
</tr>
<tr>
<td>Freeze-dried lactose [89]</td>
<td>22–23</td>
<td>54, 66, 76</td>
<td>Mixture of α-lactose monohydrate, anhydrous β-lactose, and anhydrous crystals of α- and β-lactose in molar ratios of 5:3 and 4:1</td>
</tr>
<tr>
<td>Spray-dried lactose [96]</td>
<td>25</td>
<td>55</td>
<td>Mixture of α-lactose monohydrate and anhydrous β-lactose</td>
</tr>
<tr>
<td>Spray-dried lactose [118]</td>
<td>Room temperature</td>
<td>75</td>
<td>Mixture of α-lactose monohydrate and anhydrous β-lactose</td>
</tr>
<tr>
<td>Spray-dried lactose [87]</td>
<td>25</td>
<td>53, 65, 75, 85</td>
<td>Mixture of α-lactose monohydrate and anhydrous β-lactose</td>
</tr>
<tr>
<td>Spray-dried lactose [38]</td>
<td>25</td>
<td>57, 75, 84, 100</td>
<td>Mixture of α-lactose monohydrate and anhydrous β-lactose</td>
</tr>
<tr>
<td>Spray-dried lactose [89]</td>
<td>22–23</td>
<td>54, 66, 76</td>
<td>Mixture of α-lactose monohydrate, anhydrous β-lactose, and anhydrous crystals of α- and β-lactose in molar ratios of 5:3 and 4:1</td>
</tr>
</tbody>
</table>
### TABLE 2.8 (cont.)
Crystal Forms of Lactose Found in Plasticized Lactose and Lactose-Containing Products Stored at Various Temperatures (T) and Relative Humidities (RH)\(^a\)

<table>
<thead>
<tr>
<th>Material [Ref.]</th>
<th>T (°C)</th>
<th>RH (%)</th>
<th>Crystal Forms of Lactose Present</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lactose in model systems</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Freeze-dried lactose–pullulan (3:1) [77]</td>
<td>25</td>
<td>54, 64</td>
<td>Mixture of (\alpha)-lactose monohydrate, anhydrous crystals of (\alpha)- and (\beta)-lactose in a molar ratio of 5:3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>75</td>
<td>Mixture of (\alpha)-lactose monohydrate, anhydrous crystals of (\alpha)- and (\beta)-lactose in a molar ratio of 5:3, anhydrous (\beta)-lactose (traces)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>84</td>
<td>Mixture of (\alpha)-lactose monohydrate, anhydrous (\beta)-lactose, and anhydrous crystals of (\alpha)- and (\beta)-lactose in a molar ratio of 5:3</td>
</tr>
<tr>
<td>Freeze-dried lactose–pullulan (2:1) [77]</td>
<td>25</td>
<td>54, 64, 75, 84</td>
<td>Mixture of (\alpha)-lactose monohydrate, anhydrous crystals of (\alpha)- and (\beta)-lactose in a molar ratio of 5:3</td>
</tr>
<tr>
<td>Freeze-dried lactose–pullulan (1:1) [77]</td>
<td>25</td>
<td>64, 75, 84</td>
<td>Mixture of (\alpha)-lactose monohydrate, anhydrous crystals of (\alpha)- and (\beta)-lactose in a molar ratio of 5:3</td>
</tr>
<tr>
<td>Freeze-dried lactose–sucrose–invertase [65]</td>
<td>24</td>
<td>54, 66, 76</td>
<td>(\alpha)-Lactose monohydrate (mainly), anhydrous crystals of (\alpha)- and (\beta)-lactose in a molar ratio of 5:3 (traces)</td>
</tr>
<tr>
<td>Freeze-dried lactose–sucrose–invertase–carrageenan [65]</td>
<td>24</td>
<td>66, 76</td>
<td>(\alpha)-Lactose monohydrate (mainly), anhydrous crystals of (\alpha)- and (\beta)-lactose in a molar ratio of 5:3 (traces)</td>
</tr>
<tr>
<td><strong>Lactose in milk products</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Freeze-dried solution made from spray-dried skim milk [96]</td>
<td>25</td>
<td>55</td>
<td>(\alpha)-Lactose monohydrate</td>
</tr>
<tr>
<td>Freeze-dried skim milk [96]</td>
<td>25</td>
<td>55</td>
<td>Anhydrous mixture of (\alpha)- and (\beta)-lactose in a molar ratio of 5:3</td>
</tr>
<tr>
<td>Freeze-dried skim milk [75]</td>
<td>24</td>
<td>54, 66</td>
<td>Anhydrous mixture of (\alpha)- and (\beta)-lactose in a molar ratio of 5:3</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>76, 86</td>
<td>Anhydrous mixture of (\alpha)- and (\beta)-lactose in molar ratios of 5:3 (mainly) and 4:1, stable anhydrous (\alpha)-lactose, traces of (\alpha)-lactose monohydrate (at 86% RH)</td>
</tr>
</tbody>
</table>
crystals with α- and β-lactose in a molar ratio of 5:3, and anhydrous β-lactose. The estimated percentages of total crystallinity of the sample for these crystal forms are 50, 33, and 17%, respectively. Drapier-Beche et al. [101] used the areas of three selected peaks and total area in the XRD pattern to quantify the ratio of α-lactose monohydrate and anhydrous β-lactose in freeze-dried lactose that was stored at various relative humidities at 20°C.

### 2.6 SUMMARY AND CONCLUSIONS

Mono- and disaccharides have quite similar molecular structures, but there are significant differences in their physicochemical and functional properties which may be due to, for example, the presence of various anomeric forms, conformational isomerism, and a tendency toward hydrogen bonding. Crystalline and amorphous mono- and disaccharides behave in a very different way when they are stored under various storage conditions. Key factors for their stability are temperature and water content. Even small water contents cause

<table>
<thead>
<tr>
<th>Material [Ref.]</th>
<th>T (°C)</th>
<th>RH (%)</th>
<th>Crystal Forms of Lactose Present</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spray-dried skim milk [119]</td>
<td>0–37</td>
<td>75</td>
<td>α-Lactose monohydrate</td>
</tr>
<tr>
<td>Skim milk powder [101]</td>
<td>20</td>
<td>43</td>
<td>Anhydrous β-lactose</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>53</td>
<td>Anhydrous β-lactose and α-lactose monohydrate</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>59</td>
<td>α-Lactose monohydrate</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>75</td>
<td>α-Lactose monohydrate</td>
</tr>
<tr>
<td>Spray-dried whole milk [90]</td>
<td>60</td>
<td>—</td>
<td>Anhydrous β-lactose</td>
</tr>
<tr>
<td>Spray-dried whole milk [119]</td>
<td>37</td>
<td>&lt;20</td>
<td>Anhydrous β-lactose</td>
</tr>
<tr>
<td></td>
<td>0–37</td>
<td>75</td>
<td>α-Lactose monohydrate</td>
</tr>
<tr>
<td>Spray-dried whole milk containing 28, 47, and 68% lactose [120]</td>
<td>20–22</td>
<td>77</td>
<td>α-Lactose monohydrate</td>
</tr>
<tr>
<td>Spray-dried whey and partially demineralized whey [118]</td>
<td>Room temperature</td>
<td>75</td>
<td>α-Lactose monohydrate</td>
</tr>
</tbody>
</table>

* Detected using XRD.
FIGURE 2.3 Identification of crystal form in freeze-dried lactose stored at relative humidity of 44% at 24°C for 25 days. (Data from Jouppila et al. [88].)
drastic changes in amorphous sugars, whereas crystalline sugars are stable until the water content is high enough to cause solubilization. An increase in temperature causes plasticization of amorphous sugars above their glass transition temperature, whereas crystalline sugars are stable until their melting temperatures are attained. Thus, various changes may occur in amorphous sugars in storage conditions under which crystalline sugars are stable. That is why it is very important to know the properties of amorphous sugars and the kinetics of changes occurring in amorphous sugars in order to predict their behavior during processing (dehydration and agglomeration) and during storage of food and pharmaceutical products containing amorphous sugars.

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3 Health Aspects of Mono- and Disaccharides

Anne Raben and Kjeld Hermansen

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3.1 INTRODUCTION

Recently, the World Health Organization issued a new set of revised dietary recommendations [1]. According to these, the intake of free sugars should be less than 10 energy percent (E%), the rationale being that higher intakes of free sugars threaten the nutrient quality of diets by providing significant energy without specific nutrients, thereby promoting a positive energy balance. The sugar industries protested intensely against these recommendations; however, although the industry possesses much knowledge and information on their products, independent researchers and authorities must set public health recommendations after a thorough perusal of all available data and facts. The limit of 10 E% sugar is neither new nor revolutionary; in fact, most dietary guidelines state that added sugars should be limited to 10 E% for heterogeneous population groups (e.g., *Nordic Nutrition Recommendations* [2]).

It has been suggested that sugar is a causative factor of many diseases, such as obesity, dental caries, diabetes mellitus, myocardial infarction, dyspepsia, peptic ulceration, and delinquency [3–6]. Since these claims were first stated in the 1960s, several decades have passed and much research has been done. Some of these statements have proven correct, while others have not, and as a whole a more varied picture has emerged. Experts and researchers, however, are still debating the role of sugars in various disorders, and more research is needed.

In this chapter, we present an overview of the evidence available today. We do not intend to cover all of the nutritional aspects of sugars but instead have focused primarily on the major diseases of obesity, type 2 diabetes, and atherosclerosis. Furthermore, the importance of sugars in micronutrient deficiency, dental caries, and other conditions (e.g., the immunological defense system) is described briefly.

3.2 SUGARS AND OBESITY

3.2.1 BACKGROUND

The average prevalence of obesity in Europe is now 15 to 20%, with increasing rates in most countries [7]. This translates to at least 60 million Europeans. If considering both the overweight and obese populations, the prevalence is as high as 50 to 65%, or at least 200 million Europeans. In the United States,
the prevalence of overweight and obesity is 61%, and the figure for obesity alone is as high as 27%. Also, childhood obesity is increasing at an alarming pace. Obesity is the most important risk factor for type 2 diabetes known, but obesity also gives rise to other serious complications, such as cardiovascular diseases, hypertension, and certain cancers (particularly in breast and colon), as well as psychosocial problems (e.g., depression, anxiety, loneliness, discrimination, mobbing). Further health consequences of obesity include dyslipidemia, insulin resistance, osteoarthritis, sleep apnea, asthma, lower back pain, gallbladder disease, reproductive hormone abnormalities, polycystic ovarian syndrome, impaired fertility, and childbirth complications. Taking action against the current obesity pandemic is therefore most important. A major approach is to initiate changes in food intake and eating behavior.

Weight-stable obesity is characterized not only by abnormally increased fat stores but also by increased fat-free mass (FFM). The higher FFM of obesity results in higher levels of total energy expenditure, due to both an increased basal metabolic rate and increased energy expenditure for a given physical activity. Thus, FFM is strongly and positively correlated to energy expenditure (Figure 3.1), and it is the most important determinant and predictor of individual energy requirements. The size of the FFM can account for 70 to 90% of the variability in energy expenditure between subjects [8,9]. The higher energy expenditure due to overweight and obesity suggests that, in order to avoid weight loss, obese subjects must maintain a higher energy intake than
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non-obese subjects; consequently, the key question may be whether dietary composition (in particular, an increased sugar content) promotes excessive energy intake and hyperphagia in susceptible individuals. This can be examined by epidemiological surveys, by physiological studies on appetite control and energy balance, and by dietary intervention studies.

3.2.2 Epidemiological Studies

3.2.2.1 Methodological and Analytical Pitfalls

When reviewing the literature on cross-sectional surveys and longitudinal studies, a number of common methodological and analytical flaws can be identified. These must be taken into consideration in order to achieve a consistent picture of the relationship between dietary sugar and body weight. A source of possible error, more important than nonrepresentative sampling, lies in the difficult task of self-observation and reporting of food intake, as well as of body weight and height. Undoubtedly, errors of this kind have resulted in an exaggerated range of individual variation in the response data and have therefore increased the standard errors of group means. If the response and translating errors are randomly distributed, however, the group means should still be useful measures and permit meaningful differentiation among the groups. Knowing the difficulties associated with the process of gathering valid information about food intake in the overweight and obese, one should establish a number of conditions to be fulfilled before accepting a study as valid.

It is now well established that a major pitfall is the systematic underreporting of energy by overweight and obese individuals, which has been clearly demonstrated by simultaneous measurement of free-living energy expenditure by the doubly labeled water method and of energy intake [10–12]. Prentice et al. [13] found that obese women underreported their energy intake by 30%, and others have reported similar figures [14]. It is not known whether this underreporting is selectively targeted at sweets and high-fat foods, such as cakes, ice cream, and chocolate, or if all macronutrients are equally underreported (Figure 3.1). One way to circumvent this uncertainty is to exclude studies in which substantial underreporting by overweight and obese subjects is evident from the analysis. These studies can be identified by a lack of positive relation between body fatness and energy expenditure. The same procedure should be followed when addressing the question of whether diet composition plays a role for type 2 diabetes, not least because the majority (80 to 90%) of these patients are overweight or obese.

It is also important in the testing of the relationship between sugars and body fatness that dietary sugar content expressed in E% is used to examine possible associations. When intakes are expressed in grams per day, a positive correlation will occur even if the subjects compared are consuming the same diet. This apparent relationship is due to the fact that subjects with high
energy requirements consume higher amounts of all nutrients compared to subjects with low energy requirements [15].

Besides underreporting of energy intake, overweight and obese subjects have a larger propensity to underreport their weight and to overreport their height [16,17]. This has a large influence on the body mass index (BMI) and has been shown to produce significant errors in estimating the prevalence of overweight and obese subjects; therefore, the investigators should measure weight and height, not the subjects themselves, or appropriate corrections should be made [18].

### 3.2.2.2 Cross-Sectional Studies

In valid cross-sectional studies where obvious underreporters are omitted, case-control analyses of dietary composition in obese vs. nonobese subjects consistently show that obese individuals consume a diet with a higher fat and lower carbohydrate content than do the nonobese. The diets of obese groups have been found to be 5 to 8 fat E% higher than those for the control groups. Clearly, a biological marker of habitual macronutrient intake is necessary, but no good ones exist. A proxy for dietary macronutrient composition can indirectly be obtained by measuring substrate oxidations, because the oxidative pattern seems to be relatively undisturbed by changes in dietary fat content in the first 24 hours. Using a 24-hour calorimeter, oxidative fat energy in the overweight and obese was found to be higher than in normal-weight controls (40.2 vs. 36.0%, \( p < 0.02 \)) [19]. Unfortunately, this method only provides information about total fat and carbohydrate intakes.

Larger population studies have shown that as sugar intake (expressed in E%) increases fat intake decreases, and *vice versa* [20,21]. This phenomenon has been referred to as the “fat–sugar seesaw.” A number of cross-sectional population studies have also demonstrated that a higher sugar or sucrose intake (in E%) is related to a lower body weight or BMI, and *vice versa* [22–31]. This indicates that a low intake of sugars (or sucrose) may produce overweight and obesity and that a high sugar intake may prevent weight gain. In some studies, the associations have disappeared when obvious misreporters have been excluded [28]. There are also some indications that differences exist between different genders and age groups; thus, stronger correlations have been found for men than for women in some studies [27,28,30], and in one study age was the strongest predictor of sugar intake, followed by BMI and energy intake [29].

The form in which sugar is consumed may also influence the results. Data from the past few years indicate that sugar in drinks produces increased energy intake and weight gain in the long term (see below). This finding is supported by data from, among others, the NHANES-III study of 2- to 19-year-old children which showed that overweight children had a significantly higher percent energy intake from soft drinks compared with normal-weight children [32].
Still, cross-sectional studies can only give a momentary picture of the situation and cannot reveal what produced the actual overweight. In this regard, prospective, longitudinal studies are more informative, and these therefore should be given more weight in the line of evidence.

### 3.2.2.3 Prospective Studies

A list of prospective studies that have examined the association between dietary sugar and change in BMI or body weight is given in Table 3.1. The largest study was comprised of 17,369 European men and women and had an average follow-up time of 2.2 years [33]. Here, it was observed that a high intake of sweet foods (chocolate, ice cream, sucrose) predicted weight gain in men (odds ratio = 1.48) but not in women. Also, soft drinks predicted weight gain, again only in men, but the odds ratio was only 1.03. It should be noted that this
study did not differentiate between sugar-sweetened or artificially sweetened soft drinks, water, or mineral water. Similar results regarding sweet foods were found in a 2-year follow-up study of 3552 American men and women [34]. In this study, sweet foods were defined as doughnuts, cakes, ice cream, and frozen deserts — products with a high content of both sugar and fat. A recent study in 548 children (12 years old) showed that the intake of sugar-sweetened drinks increased the risk of weight gain over 19 months [35]. For every extra sugar-sweetened soft drink, the risk of becoming obese increased 60% (BMI ≥ 30.0 kg/m²). In contrast, a smaller study in 465 men and women from New England showed no associations between sucrose intake and weight gain over 4 years [36].

To summarize, these five prospective studies showed a positive or no associations between the intake of sweet foods or drinks and weight gain. Sweet foods included both sugar-rich and sugar/fat-rich combined foods. Overweight subjects may regard sugar/fat-rich combined foods as being particularly tasty; therefore, it is likely that these foods (often snacks) contribute to weight gain due to their high energy density [37]. In one study, the group of soft drinks included several kinds of soft drinks, both with and without added sugar [33], so the interpretation is therefore not unambiguous. Another study, however, demonstrated a positive association between the intake of sugar-sweetened soft drinks and weight gain in children [35]. This is the first study with a longitudinal design that has shown that sugary drinks may be fattening, and it constitutes a milestone in the debate on sugar and obesity.

3.2.3 Physiological Studies

For weight maintenance, an energy balance must persist; that is, energy intake must equal energy expenditure. Dietary composition probably influences energy intake more than energy expenditure, although diet-induced thermogenesis may vary slightly according to macronutrient composition [38]. Evidence also exists that a macronutrient balance is important to obtaining an energy balance. This means that the intake of every single macronutrient — carbohydrate, protein, fat, and alcohol — should match the oxidation of the same macronutrient [38]. Macronutrients compete with each other in an oxidative hierarchy with the order: alcohol > protein > carbohydrate > fat. A high oxidation rate may promote satiety; therefore, a satiety hierarchy of the same order has been proposed [38]. Another view, however, is that energy density rather than macronutrient-specific mechanisms determines energy intake [39]; hence, overeating may be encouraged by energy-dense foods, which may consist of both fat and sugars. The question is which position do sugars (or sucrose) take? Do they promote excessive intake by being palatable or energy dense, or do they suppress energy intake through carbohydrate-induced satiating mechanisms — increased hepatic glycogen stores, oxidation in the peripheral tissues, or satiating hormones, such as gastric inhibitory polypeptide (GIP), glucagon-like peptide-1 (GLP-1), and insulin?
3.2.3.1 Sugars and Appetite Regulation

Sugar, in general, increases palatability, and increased sugar in the diet could therefore stimulate energy intake and cause overeating. Some studies indicate, however, that sweet carbohydrates, including sucrose, exert a suppressing effect on appetite for a limited period after consumption [40,41], while others have not found this effect [42–44]. The latter could be due to the use of quite small preloads of 20 to 40 g given in the form of a sweetened drink. It has been demonstrated that the ingestion of ≥50 g sugar (836 kJ) within 20 to 60 minutes of a meal will result in a reduced mealtime food intake, indicating that there may be a necessary energy threshold for detection by food intake control mechanisms [45]. Other researchers claim that simple sugars do not possess the same satiating capacity as complex carbohydrates [46].

Acute studies of energy intake or expenditure measured over a few hours or a day are, however, not that useful when disclosing the effect of sugars on longer term body weight regulation. Appetite regulation and macronutrient balance will not be reached until some days on a specific diet have passed, and an effect on body weight cannot be expected until after a few weeks [47,48].

In a 14-day study on normal-weight subjects, three different diets were compared with regard to appetite and *ad libitum* energy intake. One diet was rich in starch, one in sucrose, and one in fat [49]. Subjective appetite ratings showed that on the sucrose diet subjects felt more full and had less of a desire to eat compared with the fat diet, and they felt more satisfied on the sucrose diet compared with both the fat and starch diets. Despite this, *ad libitum* intake was not lowest on the sucrose diet but on the starch diet, and body weight was stable on the sucrose diet whereas it decreased on the starch diet. This could be related to quite large amounts of sugary drinks (fruit syrup and soft drinks) on the sucrose diet or to the higher dietary fiber content on the starch diet. Palatability ratings were highest on the sucrose diet, which may also have promoted energy intake. Lawton et al. [50] compared the effect of different types of snacks (± sweet or fat) on energy intake and body weight for 21 days. They found that subjects consumed more energy per day from a sweet snack than from a non-sweet snack and that most energy was consumed from a combined high-fat/sweet snack. Although fat energy percent in the diet decreased with the low-fat snacks, no differences between total energy intake and body weight were observed after 21 days.

3.2.3.2 Sugars and Energy Expenditure

Another factor to consider is carbohydrates and sugars as stimulators of energy expenditure (EE), an effect that could counteract a slight increase in energy intake. Although this issue has not been fully elucidated, some studies point
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to the carbohydrate content of the diet as an important stimulator of energy expenditure. Despite a weight loss during 20 weeks on an *ad libitum* low-fat, high-carbohydrate diet, energy intake increased by 19% compared to a control group consuming a high-fat diet [51]. An analysis of physical activity level could not disclose any change in daily activity level, which suggests that other components of energy expenditure were affected [51].

Another line of evidence comes from a study comparing diet composition and the resting metabolic rate (RMR) of vegetarians and nonvegetarians [52]. Vegetarians reported a greater intake of carbohydrates than nonvegetarians (62 vs. 51%), but no difference was found in total energy intake, body composition, or aerobic capacity. Vegetarians exhibited an 11% higher RMR, which could be explained by their higher sympathetic nervous system (SNS) activity due to the higher carbohydrate intake [52].

Lean and James [53] reported that the 24-hour energy expenditures in post-obese subjects was highly sensitive to a high-carbohydrate diet, as this diet had a stimulatory effect that could not be found in a control group. Post-obese women have previously been found to have an 8% higher 24-hour EE than a control group when consuming a low-fat diet, an effect that could be explained by higher SNS activity [49]. In a subsequent study, a dose–response relationship was established between dietary carbohydrate content and 24-hour EE in post-obese women, while no such relation could be established in controls [55]. This different responsiveness to carbohydrates may be due to a genetic susceptibility of obesity-prone subjects.

Regarding the different types of carbohydrates, an increased intake of sucrose has been shown to stimulate thermogenesis compared with glucose or starch in an acute situation [56–58]. This can probably be explained by the fructose moiety of sucrose, due to the increased cost of converting fructose to glucose in the liver [56].

Only a few studies have looked at the effect of the long-term intake of different carbohydrates on energy expenditure. One was a 14-day *ad libitum* study in normal-weight, post-obese subjects and matched controls [49]. Here it was found that 24-hour energy expenditures were 3% and 4.5% higher on a low-fat, sucrose-rich diet compared with a high-fat or a low-fat, starch-rich diet, respectively, mainly due to an increase in energy expenditure in the post-obese subjects. Also, noradrenaline and adrenaline were increased on the high-sucrose diet, indicating a stimulatory effect of this diet on the sympathetic nervous system.

Still, in the long-term CARMEN trial, no differences in 24-hour energy expenditure were observed after 6-month *ad libitum* diets high in simple carbohydrates, high in complex carbohydrates, or high in fat [59]. The lack of difference could be related to the small sample size (*n* = 7 to 9 per group), the specific study group (overweight and obese), or perhaps adaptation to the diets in the long term.
3.2.3.3 Sugars and *De Novo* Lipogenesis

The storage capacity of carbohydrates in the human body is normally small, averaging 400 to 500 g glycogen in the liver and muscle tissue. If more than this amount is consumed, the surplus must be converted to heat or to fat by *de novo* lipogenesis (DNL). In rats, *de novo* lipogenesis is an extensively used biochemical pathway, but its role in humans may not be as important. Normally, the human diet is ample in dietary fat, which decreases the necessity to convert carbohydrates to fat in order to store fat in the adipose tissue. On the basis of this and previous *in vitro* studies, the activity of lipogenic enzymes has been considered to be very low, although some studies have suggested that DNL in humans could be stimulated after 3 to 6 days on a low-fat, very-high-carbohydrate diet [60].

The significance of hepatic DNL *in vivo* has, however, been difficult to assess due to methodological limitations. Thus, indirect calorimetry, which was used before, can only quantify net DNL (total body fat oxidation – total DNL); however, in the past decade new techniques have been developed that have contributed to a greater understanding of the *in vivo* processes, such as the stable-isotope tracer technique and mass isotopomer distribution analysis, as well as endogenous dilution of linoleate, an essential fatty acid that cannot be synthesized *de novo* [61]. Using these methods, it was recently shown that 5 days on a high-carbohydrate, low-fat diet increased fractional *de novo* lipogenesis to 13%, which was 5 to 6 times higher than when a high-fat, low-carbohydrate diet was consumed. Also, triacylglycerol increased in a manner correlating to the increase in *de novo* lipogenesis [61]. This was found in normoinsulinemic lean and hyperinsulinemic obese subjects. Some results have also indicated that simple sugars may stimulate DNL more than complex carbohydrates [62,63]. Overall, it seems that a very high carbohydrate content in the diet (>75 E%) is needed to induce a significant increase in DNL in humans.

3.2.3.4 Sugars and Body Weight

Several dietary intervention trials have shown that the recommended high-carbohydrate, high-fiber diet that is low in fat and energy density *ad libitum* causes spontaneous weight loss, especially in overweight subjects [54,64–66]. Among the most convincing studies are those aimed at reducing dietary fat content in order to improve levels of blood lipids or to prevent breast cancer. A side effect found in these studies was that body weight decreased as the fat energy percent decreased. According to the metaanalyses recently published, it was shown that a reduction of 10% in the proportion of energy from fat is associated with a reduction in body weight of 2.8 kg over 6 months [64–66]. These weight losses may seem small, but when compared with the gradual increase in body weight many people now experience over time, a weight loss
of even a few kilograms over 6 months is important, especially when no calorie restriction is involved in obtaining this weight loss. Furthermore, the recommended diet has been shown to improve weight maintenance and quality of life compared with a conventional calorie-counting diet [67]. A spontaneous reduction in energy intake due to a low energy density (great volume) and a high fiber intake is probably a major reason why such a diet decreases and helps maintain body weight in the long term.

The question, then, is whether sugars act like starch in this respect or whether, because of a possibly higher energy density and palatability, they assume an intermediate position between fat and starch. The effect of different types of carbohydrates on long-term body weight regulation, however, has not been investigated to as large an extent as the recommended diet per se, but some longer term studies exist [49,68].

Intervention studies with a weight-maintaining or weight-loss design cannot disclose how sugars affect appetite and body weight in a real-life situation; therefore, it is relevant to focus mainly on studies using an ad libitum design. The only large-scale, long-term, randomized, controlled trial is the CARMEN (Carbohydrate Ratio Management in European National diets) multicenter trial, which involved a total of 316 overweight subjects in 5 different countries [68]. Here it was found that 6 months’ ad libitum intake of low-fat diets rich in either simple or complex carbohydrates reduced body weight and fat mass by 1.6 to 2.4 kg compared with a higher fat, control diet, with no significant differences between the simple and complex carbohydrate diets. Although this is a study with great statistical power, and therefore should be given much emphasis, it is relevant to look at other published studies. A compilation of these is given in Figure 3.2.

A total of 12 intervention studies have been published, 2 of them comparing sugar with artificial sweeteners (Figure 3.2). The picture that emerges suggests that when a sugar-rich diet is compared with a fat-rich or habitual diet (a total of 7 studies), the fat-rich diet produces a small weight gain compared with the sugar-rich diet; however, when a sugar-rich diet is compared with a starch-rich diet (a total of 3 studies), the starch-rich diet produces a weight loss compared with the sugar-rich diet. When sugar is exchanged for artificial sweeteners, a weight gain is seen with sugar compared with artificial sweetener. The latter 2 studies are, however, characterized by an experimental diet consisting largely of drinks [69,70]. As mentioned earlier, fluid calories are not believed to be very satisfying, and this may explain the weight gain observed in these 2 studies.

### 3.2.3.5 Fluids vs. Solid Foods

Because calories from drinks are likely to be less satiating compared with calories from solid foods, it is therefore easier to obtain an exaggerated energy intake when drinking compared with eating the calories. In one crossover
study, it was found that 1880 kJ/d from soft drinks increased body weight after 4 weeks whereas the weight was unchanged when the same calories came from jelly beans [71]. A metaanalysis of 42 studies also showed that compensation was much less precise when fluids were ingested as compared to solid foods [72]. The suggested mechanisms are primarily related to fewer satiating-producing signals after the intake of fluid calories, more specifically related to less chewing, less stimulation of the cephalic phase, faster gastric emptying, less contact with receptors in the intestinal tract, and less of an increase in satiating hormones. Finally, it could be that higher energy expenditures after solid vs. fluid foods contribute to a lower propensity for gaining weight when consuming primarily solid foods [73].

3.2.3.6 Glycemic Index, Appetite, and Body Weight Regulation

Classifying carbohydrates according to their chemical composition (simple vs. complex carbohydrates, sucrose or starch) has been criticized from a physiological point of view; therefore, considering the glycemic and insulinemic impacts of carbohydrates is recommended when trying to evaluate the health effects of carbohydrates (see Section 3.3). It has been suggested that low-glycemic-index (GI) foods increase satiety and decrease fat deposition and weight gain compared with high-GI foods [74,75]; however, controversies still exist [76,77]. Recently, a systematic review of published human intervention

![Body weight changes on ad libitum sugar-rich diets](image-url)
studies compared the effects of high- and low-GI foods or diets on appetite, food intake, energy expenditure, and body weight [78]. Since then, a few more studies have been published. In a total of 31 short-term studies (<1 d), low-GI foods were associated with greater satiety or reduced hunger in 15 of them, whereas reduced satiety or no differences were seen in the other 16 studies. Low-GI foods reduced *ad libitum* intake in 7 studies but not in 8 other studies. In 20 longer term studies (<6 months), weight loss was seen on a low-GI diet in 4 studies and on a high-GI diet in 2 studies, but no differences were seen in 16 studies. The average weight loss was 1.5 kg on low-GI diets and 1.6 kg on high-GI diets. When looking only at *ad libitum* studies, 4 such studies have lasted for 2 to 4 months [49,79–81]. From these studies, no differences can be seen in average weight changes between low- and high-GI diets (Figure 3.3).

### 3.2.4 Conclusion

Solid evidence suggests that a change from the typical Western high-fat diet to a low-fat, high-carbohydrate, high-fiber diet may cause spontaneous weight loss, in particular in overweight and obese subjects. To make this diet more palatable and increase adherence, the diet may include sugars (up to 10 E% as recommended); however, sugary drinks should be avoided. The glycemic index has, so far, not been proven relevant to body weight regulation.
3.3 SUGARS AND TYPE 2 DIABETES MELLITUS

3.3.1 BACKGROUND

Type 2 diabetes mellitus (T2DM) is the most common metabolic disease, and the number of diabetic individuals is increasing worldwide. It is claimed that we are experiencing a true diabetes pandemic. At present, the number of diabetic patients in the world is estimated to range between 150 and 200 million, a number that has been calculated to reach 300 million diabetic patients in 2025. Recent statistics from the Centers for Disease Control and Prevention indicate that nearly two thirds of American adults are overweight, more than 30% are frankly obese, and nearly 8% are diabetic. It is estimated that the number of individuals in the United States with diagnosed diabetes will increase by 165% between 2000 and 2050, with the fastest increases occurring in older and minority subpopulations, such as Hispanics [82]. The estimated lifetime risk of developing diabetes for individuals born in 2000 is 33% for males and 39% for females [83]. Type 2 diabetes is characterized by decreased insulin sensitivity (i.e., insulin resistance and impaired first-phase insulin secretion). Type 2 diabetes is a progressive disease with declining beta cell function and insulin secretion during the course of the disease. Over 75% of newly diagnosed type 2 diabetic patients are obese. Being overweight or obese is regarded as the major cause of type 2 diabetes in genetically predisposed individuals. The relationship between dietary sugars and type 2 diabetes should therefore be considered when examining the importance of sugars in weight control.

3.3.2 DIABETIC COMPLICATIONS AND GLYCEMIC CONTROL

Diabetes is the leading cause of blindness, renal failure, and amputation in the United States. The microvascular complications can be slowed or prevented with optimal glycemic control in both type 1 [84] and type 2 diabetes [85]. Accompanying diabetes is a concomitant two- to fourfold excess risk for cardiovascular disease [86,87]. The relationship between diabetes and cardiovascular disease (CVD) is so clear that the American Diabetes Association (ADA) has identified diabetes as a CVD, while the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) has come to the conclusion that diabetes should be considered a coronary heart disease (CHD) risk equivalent. Hyperglycemia is the driving force in microvascular complications of diabetes, while the macrovascular complications are due to an exposure of the vasculature to a frontal assault by hyperglycemia, hypertension, dyslipidemia, inflammation, and impaired fibrinolysis [86,87]. Individuals diagnosed as having diabetes have a large reduction in life expectancy; for example, if diagnosed at age 40 years, men will lose 11.6 and women 14.3 life-years, respectively [83].
Because hyperglycemia plays a critical role in the complications of diabetes, how do fasting hyperglycemia and postprandial glycemia contribute to the overall hyperglycemia reflected in HbA1C values? The postprandial glycemic excursions play a major role in the metabolic disequilibria of patients suffering from mild or moderate hyperglycemia [88], whereas fasting hyperglycemia appears to be a main contributor to the overall diurnal hyperglycemia in poorly controlled diabetic patients. The role of postprandial glucose elevations decreases as patient’s progress toward poor diabetic control [88].

Recent studies have documented the importance of postprandial hyperglycemia per se for all-cause and cardiovascular mortality [89]. The blood glucose concentration 2 hours after a standard glucose tolerance test is taken as a surrogate measure of meal-induced hyperglycemia. The DECODE study [89], an analysis of more than 20 European studies, demonstrated that increased rates of mortality and morbidity are associated with high blood glucose levels in diabetic patients. A high blood glucose concentration appears to be damaging to the endothelium through a variety of mechanisms [90]. High glucose levels interfere with vasodilation by inhibiting nitrous oxide synthase and reducing the production of nitrous oxide. Excessive postprandial hyperglycemia is also directly toxic to the endothelium by increasing protein glycation (advanced glycated end product [AGE]) [90].

3.3.3 SUGAR INTAKE AND THE RISK OF DEVELOPING DIABETES

Dietary prescriptions in the early 20th century emphasized the strict restriction of carbohydrate intake and, in particular, an avoidance of sugars [91]. The restriction of sugar intake appears to have been an intuitive concept, and it was presumed that an illness defined by elevated blood sugar was almost certainly linked to the ingestion of sugars. Several animal experiments have unanimously shown that a high intake of sucrose or fructose increases insulin resistance; however, in contrast, the relatively few epidemiological studies in humans focusing on sugar consumption and the risk of type 2 diabetes have been inconclusive. Cross-sectional studies have demonstrated the same consumption of sucrose and fructose in people with and without diabetes [92] as well as a reduced intake of refined sugar in those with diabetes [93]. A cross-sectional study among Japanese migrants from Hawaii showed a positive association between the intake of sugar and the prevalence of diabetes [94], but Colditz et al. [95] did not detect any association between sucrose intake and the prevalence of diabetes in either slender or overweight women. In the quintile of women with the highest sucrose consumption, Meyer et al. [96] found an inverse relationship with the development of diabetes (relative risk [RR] = 0.81), while a positive association was observed between the intake of both fructose and glucose and the development of diabetes. In a large cohort of women (38,480) in the Women’s Health Study, initially healthy postmenopausal women were followed prospectively for an average of 6 years, and no
relationship was observed between sugar intake and risk of type 2 diabetes [97]. Compared with the lowest quintile of sugar intake, the relative risk and 95% confidence interval were 0.84 (0.67 to 1.04) for sucrose, 0.96 (0.78 to 1.19) for fructose, 1.04 (0.85 to 1.28) for glucose, and 0.99 (0.80 to 1.22) for lactose, after adjustment for known risk factors for type 2 diabetes [97]. In conclusion, no clear association between the intake of sugars and the development of diabetes has been identified.

### 3.3.4 Glycemic Responses to Sugars

It was earlier believed that mono- and disaccharide-rich food items produced high glucose and insulin responses, while polysaccharide-rich (starchy) foods produced low and longer-lasting responses. Today, it is well established that this is not the case. Acute intervention studies have shown that starchy foods (e.g., rice, potato, bread) may produce high glucose responses, while fruits with high amounts of mono- and disaccharides (e.g., apple, orange, pear) may produce low responses [98–100]. Furthermore, different types of mono- and disaccharides may result in different glucose and insulin responses. We know that blood glucose concentration increases less after administration of sucrose compared to glucose and even less after the administration of fructose [99].

In 1981, Jenkins and co-workers [99] proposed the glycemic index (GI) as a method of assessing and classifying glycemic responses to foods. It was hoped that this would allow foods to be compared more readily. The GI was defined as the incremental area under the blood glucose response curve for the actual food expressed as a percentage of the incremental area after taking the same amount of carbohydrate as glucose:

\[
\text{Glycemic index} = \frac{\text{Blood glucose area of test food}}{\text{Blood glucose area of reference food}} \times 100
\]

In most centers, the amount of test food has now been standardized to 50 g (or 25 g for low-carbohydrate food items), and the reference food is either white wheat bread or glucose. Usually, blood glucose responses are measured for 2 or 3 hours in normal subjects and 3 hours in diabetic patients [98]. More than 1200 foods have now been tested by applying this approach [101].

The GIs of sugars and some common foods are presented in Table 3.2. Solutions of different sugars as fructose, sucrose, and lactose have been compared to glucose by, for example, Jenkins et al. [99], who found that fructose has a GI of 23, sucrose (fructose–glucose) has a GI of 61, and lactose (galactose–glucose) has a GI of 46. It should, however, be stressed that large variations in GI values exist, even for the same source of carbohydrate. Important variables that affect the glycemic responses to a test meal include intrasubject and intercenter variations found in GI responses [94]. The day-to-day variation of blood glucose responses (intraindividual variation) in type 2 diabetic...
patients expressed as the coefficient of variation constituted 15 to 19% [102,103], while the interindividual variations in type 2 diabetic patients were larger, attaining 23 to 34% [102,103]. Other important variables of influence are methods of area calculation; length of observation times; subject characteristics (e.g., gender, age, weight); type of diabetes; preprandial blood glucose level; and food factor (amount; eating time; nature of starch; content of fat, protein, and fiber; food processing and storage) [98]. For practical purposes, however, we believe that the glycemic index can be used as a guideline in choosing the proper carbohydrate-rich food item when aiming at low or high blood glucose levels.

It can be seen from the Table 3.2 that high levels of sucrose and fructose in fruits are not predictors of high GIs; in fact, rather the contrary is true, except for ripe bananas [104]. Oranges [105], apples [105], unripe bananas [104], grapefruits, and grapes all have low GIs. The GI rises slightly when the fruits are consumed as a juice rather than as whole fruit. Sucrose, too, has a GI a little above medium (61 to 67 compared to glucose). This may be compared with the higher GIs of bread, rice, or potato.

### TABLE 3.2
Glycemic Index of Common Foods

<table>
<thead>
<tr>
<th>Foods</th>
<th>GI wb</th>
<th>GI g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sucrose</td>
<td>92</td>
<td>61–67</td>
</tr>
<tr>
<td>Glucose</td>
<td>138</td>
<td>100</td>
</tr>
<tr>
<td>Fructose</td>
<td>32</td>
<td>23</td>
</tr>
<tr>
<td>Lactose</td>
<td>63</td>
<td>46</td>
</tr>
<tr>
<td>White bread</td>
<td>100</td>
<td>72</td>
</tr>
<tr>
<td>Pasta</td>
<td>46–88</td>
<td>33–64</td>
</tr>
<tr>
<td>Rice (polished)</td>
<td>68–104</td>
<td>49–75</td>
</tr>
<tr>
<td>Rice (parboiled)</td>
<td>58–78</td>
<td>42–56</td>
</tr>
<tr>
<td>Cornflakes</td>
<td>107–139</td>
<td>78–100</td>
</tr>
<tr>
<td>Potatoes</td>
<td>66–120</td>
<td>48–87</td>
</tr>
<tr>
<td>Beans</td>
<td>40–60</td>
<td>30–43</td>
</tr>
<tr>
<td>Lentils (red, dried)</td>
<td>25–45</td>
<td>18–33</td>
</tr>
<tr>
<td>Peas (green, frozen)</td>
<td>55–74</td>
<td>40–54</td>
</tr>
<tr>
<td>Oranges</td>
<td>44–73</td>
<td>32–53</td>
</tr>
<tr>
<td>Bananas</td>
<td>43–99</td>
<td>31–72</td>
</tr>
<tr>
<td>Apples</td>
<td>40–57</td>
<td>29–41</td>
</tr>
</tbody>
</table>

* GI has been calculated compared to white bread (GI wb) or glucose (GI g). The values are given as a mean or range. GI wb/GI g = 1.38.
3.3.5 Absorption of Sugars

The reason why the GI is lower for sucrose and fruits than for many starchy foods relates mainly to the metabolic fate of fructose from fruits and sucrose in the human body. Fructose is absorbed across the intestinal mucosa at a slower rate than glucose, possibly by facilitated diffusion [106]. Some of the fructose is converted to glucose, lactate, or alanine by the intestinal epithelium, but 70 to 90% enters the hepatic portal vein as fructose [107–109]. Fructose is then metabolized in the liver, where fructokinase catalyzes the rapid phosphorylation of fructose into fructose-1-phosphate. The removal of fructose from the circulation is rapid and efficient, so the blood concentration of fructose usually never exceeds 1.0 mmol/l [110].

As for sucrose, this disaccharide is enzymatically hydrolyzed by sucrase in the brush border of the intestinal epithelium [111]. The resulting monosaccharides (i.e., glucose and fructose) are transported through the brush-border membrane directly, without being released into the luminal space. Glucose is transported by specific carrier systems, one of which is Na⁺ dependent [112]. When fructose and glucose are ingested simultaneously, the intestinal absorptive capacity for fructose increases; therefore, the hypothesis prevails that the disaccharidase-related transport system participates in this facilitating absorptive effect [106].

In contrast to fructose and sucrose, starch (except for the fractions now classified as resistant starch [113]) is usually fully degraded by α-amylase in the small intestine and absorbed directly as glucose into the portal blood stream. The rate of digestion and uptake may, however, vary from starch to starch.

3.3.6 Consumption of Sugars and Metabolic Control in Diabetes

The evidence from clinical intervention studies in diabetic subjects unanimously shows that the sucrose content of the diet in the short term does not affect the glycemic control more than an isocaloric amount of starch [114–121]. Bantle et al. [116] compared the postprandial glycemic response to various forms of carbohydrate (42 g separately of glucose, fructose, sucrose, potato starch, and wheat starch) that composed 25% of total energy within a mixed meal also containing protein and fat. Fructose ingestion led to a lower postprandial glycemic response in those with diabetes while the other types of carbohydrate had nearly the same impact. In 12 type 1 and 12 type 2 diabetic patients, three isoenergetic diets were fed for 8 days providing 23 E% as sucrose, 21 E% as fructose, and almost all carbohydrate as starch [115]. There was no difference between sucrose and starch diets in any of the measures of the glycemic control, but the fructose diet resulted in overall lower glucose levels [115]. The same group subsequently studied 12 type 2 diabetic patients who consumed in random order two isocaloric, 55 E% carbohydrate diets for 4
Health Aspects of Mono- and Disaccharides

In one diet, 19 E% was derived from sucrose and in the other <3 E% was derived from sucrose and carbohydrate primarily came from starch. No significant differences were noted between the study diets in mean plasma glucose or triglycerides [118]. Abraira et al. [117] studied 18 type 2 diabetic subjects on a diet with 50 E% carbohydrate and 120 g sucrose for 10 days as inpatients. They were then randomly assigned diets of similar composition with either 220 g sucrose or <3 g sucrose daily. This large difference in sucrose intake with constant fat and carbohydrate intake did not affect glycemic or triglyceremic control in type 2 diabetic patients [117]. Malerbi et al. [119] investigated the metabolic effects of dietary sucrose and fructose in 16 type 2 diabetic patients. They were fed three isocaloric diets containing 50 to 55 E% for 4 weeks. In one diet, 19 E% was derived from sucrose, in another 20 E% was derived from sucrose, and in the control diet only 5 E% was derived from sugars, all other carbohydrates being starch. In the 4-week period, the high-sucrose and high-fructose diets did not adversely affect glycemia or lipidemia [119]. The reason why fructose has a lower glycemic response is attributable to a predominantly hepatic uptake of fructose with very limited release of endogenous glucose production. Although there is potentially a favorable metabolic postprandial effect of fructose on postprandial blood glucose, large amounts of fructose may adversely increase low-density lipoprotein (LDL) cholesterol [122].

In the American Diabetes Association (ADA) evidence-based nutrition principles and recommendations for the treatment and prevention of diabetes and related complications [123], it is stated that sucrose and sucrose-containing food do not need to be restricted by people with diabetes based on a concern about aggravating hyperglycemia; however, if sucrose is included in the food/meal plan, it should be a substitute for other carbohydrate sources, or, if added, be adequately covered with insulin or other glucose-lowering medication [123,124]. On the other hand, the Diabetes and Nutrition Study Group (DNSG) of the European Association for the Study of Diabetes (EASD) recommends that, if desired, moderate intakes of sucrose may be incorporated within the diet for both types of diabetes [125]. As for the general population, DNSG recommends that the intake of sucrose not exceed 10 E% [125]. This caution expressed by the DNSG is based on the fact that no long-term studies in diabetic subjects have demonstrated that a dietary habit where the bulk of carbohydrates are derived from highly refined (processed) starchy foods rich in sucrose is compatible with good glycemic control and optimum levels of risk factors for the complications of diabetes [126]. Furthermore, an intake of diets high in sugars may be deficient in fiber and micronutrients, and a high intake of sugary beverages has been convincingly shown to relate to the subsequent risk of obesity [126]. Both the ADA and DNSG emphasize the value of selecting vegetables, fruit, and grains so the starches consumed will include adequate amounts of both fiber and micronutrients.
3.3.7 Glycemic Index, Glycemic Load, and the Risk of Type 2 Diabetes

The possibility that the high, long-term intake of carbohydrates that are rapidly absorbed as glucose may increase the risk of type 2 diabetes has long been the subject of controversy [127]. The concept of the glycemic index can be applied not only to one food but also to entire meals or overall diets. Because the amount of carbohydrate in a food or overall diet can vary, the concept of glycemic load (GL) has been introduced. GL is the amount of carbohydrate multiplied by its glycemic index. Recently, data have become available from large, long-term epidemiological studies relating dietary GI or GL to the risk of type 2 diabetes, as shown in Table 3.3 [96,128,129]. In 1986, a total of 65,173 U.S. women ages 40 to 65 years and free from diagnosed CVD and diabetes completed a detailed dietary questionnaire [128]. During 6 years of follow-up, 915 incident cases of diabetes were documented. Comparing the highest with the lowest quintile, the relative risk (RR) of diabetes and 95% confidence interval (CI) were 1.37 and 1.09 to 1.71, respectively. The glycemic load was also positively associated with diabetes (RR = 1.47; 95% CI, 1.16 to 1.86). The combination of high glycemic load and a low cereal fiber intake further increases the risk of diabetes (RR = 2.50; 95% CI, 1.14 to 5.51) when compared with a low glycemic load and high cereal fiber intake [128]. In 1986, Salmeron et al. [129] studied a cohort of 42,759 men without diabetes and

<table>
<thead>
<tr>
<th>Ref.</th>
<th>Design</th>
<th>N</th>
<th>Highest/Lowest GI</th>
<th>Relative Risk</th>
<th>Comments</th>
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</thead>
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<tr>
<td>128</td>
<td>Cohort study</td>
<td>65,759 women</td>
<td>79/64</td>
<td>1.37 for GI</td>
<td>Significant for 5th vs. 1st quintile</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.47 for GL</td>
<td>Significant for 5th vs. 1st quintile</td>
</tr>
<tr>
<td>129</td>
<td>Cohort study</td>
<td>42,759 men</td>
<td>79/65</td>
<td>1.37 for GI</td>
<td>Significant for 5th vs. 1st quintile</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.25 for GL</td>
<td>No significant association</td>
</tr>
<tr>
<td>96</td>
<td>Cohort study</td>
<td>35,988 older women</td>
<td>&gt;80/&lt;58</td>
<td>0.89 for GI</td>
<td>No significant association</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.95 for GL</td>
<td>No significant association</td>
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</tbody>
</table>
CVD who were 40 to 75 years of age. During 6 years of follow-up, 523 incident cases of diabetes were found. Comparing the highest and lowest quintiles of GI, the RR of type 2 diabetes was 1.37 (95% CI, 1.02 to 1.83), while no significant association regarding GL was found [129]. In contrast, Meyer et al. [96], in a 6-year prospective cohort study of 35,988 older Iowa women initially free of diabetes, found no statistical significant association between GI or GL and type 2 diabetes, as shown in Table 3.3. The pattern of risk across quintiles of glycemic index was inconsistent; RRs first rose to 1.22 in quintile 3 and then dropped to 0.84 in quintile 5. Interestingly, the data of Meyer et al. [96] is in accordance with the studies of Salmeron et al. [128,129] supporting a protective role for grains (particularly whole grains), cereal fiber, and dietary magnesium in the development of diabetes. An association between the glycemic index or load and the risk of developing type 2 diabetes is not yet clarified, and further research is needed.

3.3.8 Glycemic Index and Metabolic Control in Diabetes

The benefits of improving glycemic control with regard to the risk of diabetic complications are now verified [91,92]. The concept of a glycemic index was developed to provide a numeric classification of carbohydrate foods on the assumption that such data would be useful in the management of diabetes. Although logic suggests that low-GI diets should improve glycemic control, the findings of randomized controlled trials have been mixed. Some studies have shown statistically significant improvements whereas others have not. In a recent metaanalysis, Brand-Miller et al. [130] identified 14 studies involving 356 subjects. All were randomized crossover or parallel design of 12 days’ to 12-months’ duration with modification of at least two meals per day. Table 3.4 shows the studies included in the metaanalysis [131–144] as well as a more recent study [145]. The metaanalysis demonstrated that low-GI diets reduced HbA1C by 0.43% (CI, 0.72 to 0.13) over and above that produced by high-GI diets. Taking both HbA1C and fructosamine data together, the glycated proteins were reduced 7% more on the low-GI diet than on the high-GI diet.

The issue of the glycemic index has been controversial and has polarized the opinion of the experts. The ADA acknowledges that, although the use of low-GI food may reduce postprandial hyperglycemia, evidence of long-term benefit is not sufficient to recommend the general use of low-GI diets in type 2 diabetes patients as a primary strategy in food and meal planning [123,124]. In contrast, the DNSG recommends that foods with a low GI (e.g., legumes, oats, pasta, parboiled rice, certain raw fruits) should be substituted when possible for those with a high GI [125]. The FAO/WHO Expert Consultation on carbohydrates also endorsed the use of glycemic index as a means of determining optimum carbohydrate-containing foods [146]; however, the
<table>
<thead>
<tr>
<th>Ref.</th>
<th>Type</th>
<th>N</th>
<th>Study Design (High-GI vs. Low-GI Diet)</th>
<th>Diet Duration (wk)</th>
<th>Outcome (HbA1c or Fructosamine)</th>
<th>Endpoint</th>
<th>GI</th>
<th>High</th>
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<tr>
<td>Gilbertson et al. [131]</td>
<td></td>
<td>104</td>
<td>Parallel (79 vs. 77)</td>
<td>52</td>
<td>HbA1c</td>
<td></td>
<td>8.0 (1)</td>
<td>8.6 (1.4)</td>
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<tr>
<td>Giacco et al. [132]</td>
<td>Type 1 subjects</td>
<td>63</td>
<td>Parallel (90 vs. 70)</td>
<td>24</td>
<td>HbA1c</td>
<td></td>
<td>8.6 (0.9)</td>
<td>9.1 (1.4)</td>
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<tr>
<td>Lafrance et al. [133]</td>
<td></td>
<td>9</td>
<td>Crossover (99 vs. 63)</td>
<td>1.9</td>
<td>Fructosamine</td>
<td></td>
<td>2.9 (0.6)</td>
<td>3.1 (0.3)</td>
</tr>
<tr>
<td>Fontvieille et al. [134]</td>
<td></td>
<td>12</td>
<td>Crossover (90 vs. 53)</td>
<td>5</td>
<td>HbA1c</td>
<td></td>
<td>8.3 (1.4)</td>
<td>8.3 (1.5)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Fructosamine</td>
<td></td>
<td>3.41 (0.42)</td>
<td>3.88 (0.95)</td>
</tr>
<tr>
<td>Fontvieille et al. [135]</td>
<td></td>
<td>8</td>
<td>Crossover (84 vs. 65)</td>
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<td>Fructosamine</td>
<td></td>
<td>2.17 (0.68)</td>
<td>2.77 (0.59)</td>
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<tr>
<td>Collier et al. [136]</td>
<td></td>
<td>7</td>
<td>Crossover (82 vs. 69)</td>
<td>6</td>
<td>HbA1c</td>
<td></td>
<td>10 (1.2)</td>
<td>9.8 (1.5)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>GSA[4]</td>
<td></td>
<td>10.7 (5.8)</td>
<td>14.6 (5.0)</td>
</tr>
<tr>
<td>Komindr et al. [137]</td>
<td>Type 2 subjects</td>
<td>10</td>
<td>Crossover (106 vs. 70)</td>
<td>4</td>
<td>HbA1c</td>
<td></td>
<td>10.97 (1.55)</td>
<td>11.15 (2.02)</td>
</tr>
<tr>
<td>Luscombe et al. [138]</td>
<td></td>
<td>21</td>
<td>Crossover (88 vs. 60)</td>
<td>4</td>
<td>Fructosamine</td>
<td></td>
<td>3.22 (0.5)</td>
<td>3.28 (0.55)</td>
</tr>
</tbody>
</table>

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<table>
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<tr>
<th>Study</th>
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<th>Design</th>
<th>glycemia</th>
<th>HbA1c</th>
<th>Fructosamine</th>
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<td>Jarvi et al. [139]</td>
<td>20</td>
<td>Crossover (83 vs. 57)</td>
<td>3.5</td>
<td>6.7 (1.3)</td>
<td>6.9 (1.3)</td>
</tr>
<tr>
<td>Frost et al. [140]</td>
<td>51</td>
<td>Parallel (82 vs. 77)</td>
<td>12</td>
<td>3.47 (0.72)</td>
<td>3.56 (0.75)</td>
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<tr>
<td>Wolever et al. [141]</td>
<td>15</td>
<td>Crossover (87 vs. 60)</td>
<td>2</td>
<td>3.17 (0.46)</td>
<td>3.28 (0.58)</td>
</tr>
<tr>
<td>Wolever et al. [142]</td>
<td>6</td>
<td>Crossover (86 vs. 58)</td>
<td>6</td>
<td>4.56 (1.3)</td>
<td>5.12 (1.42)</td>
</tr>
<tr>
<td>Brand et al. [143]</td>
<td>16</td>
<td>Crossover (90 vs. 77)</td>
<td>12</td>
<td>7.0 (1.2)</td>
<td>7.9 (2.0)</td>
</tr>
<tr>
<td>Jenkins et al. [144]</td>
<td>8</td>
<td>Crossover (91 vs. 67)</td>
<td>2</td>
<td>7.6 (1.4)</td>
<td>7.8 (1.9)</td>
</tr>
<tr>
<td>Heilbronn et al. [145]</td>
<td>45</td>
<td>Parallel (75 vs. 43)</td>
<td>8</td>
<td>2.98 (0.45)</td>
<td>2.95 (0.45)</td>
</tr>
</tbody>
</table>

*Note:* Mean GI on each diet using bread as the reference food (GI = 100). If glucose was used as the reference food, the value was multiplied by 100/70.

- Standard deviations are in parentheses.
- HbA1c is expressed in percent (%)
- Fructosamine is expressed in mmol/l.
- Glycated albumin (GSA), expressed in percent (%), is an older measure of fructosamine.
controversy regarding the clinical significance and benefits of the glycemic index in the nutritional management of diabetes has still not been resolved [123–126,147–150].

3.3.9 Conclusion

No evidence suggests that a high intake of mono- and disaccharides is causative in the development of either type 1 or type 2 diabetes. Although the restriction of mono- and disaccharides was, until recently, a cornerstone in the treatment of both types of diabetes, more recent studies have demonstrated that a more liberal intake of sugars does not adversely affect glycemic control, and the recent dietary recommendations for diabetes now allow sucrose to amount to 10% of the total energy intake. Evidence suggests that low glycemic index diets improve glycemic control; however, controversy as to the clinical significance and benefits of the GI in the nutritional management of diabetes still exists.

3.4 Sugars and Cardiovascular Disease

3.4.1 Consumption of Sugars and Risk of Coronary Heart Disease

Coronary heart disease (CHD) remains the major cause of premature death in most affluent societies. As for most other dietary constituents, long-term intervention trial data relating sugar consumption to the development of cardiovascular disease (CVD) events are unavailable. Longitudinal cohort studies relating sugar consumption to CVD are equivocal because of the many potential confounders that cannot be adequately controlled in the analyses. The Iowa Women’s Health Study showed no relation between the intake of sweets or desserts and the risk of ischemic heart disease in 34,492 women monitored for 9 years [151]; however, some major sources of sugar were not considered. The Scottish Heart Health Study [152] of 10,359 subjects found that neither intrinsic nor extrinsic sugars were significantly associated with CVD after adjustment for other dietary variables.

A number of studies link dietary sugar with adverse changes in lipoproteins. Several studies have shown an inverse relationship between dietary sucrose and high-density lipoprotein (HDL) cholesterol [153–155]. A diet high in sucrose (>20 E%) is often associated with an elevation of plasma triglycerides [155,156]. This increase is due to both increased hepatic secretion and impaired clearance of very-low-density lipoprotein (VLDL). Fructose shifts the balance from oxidation to esterification of fatty acids, which can increase VLDL lipoprotein synthesis. In feeding studies, however, fructose has an inconsistent effect on plasma triglyceride levels, which may be related to factors such as the amount of fructose consumed, insulin sensitivity, baseline
triglycerides, and energy balance [157]. The American Heart Association [158] states that no data suggest that sugar intake per se is advantageous, and some data suggest it may be detrimental; therefore, the AHA recommends that high sugar intake should be avoided [158].

3.4.2 Glycemic Load, Glycemic Index, and the Risk of Coronary Heart Disease

Glycemic load has been found in the Nurses Health Study [159] to be an independent predictor of risk of CHD in apparently healthy women. The women in the highest quintile of GL had a twofold increased risk of myocardial infarction over 10 years compared to the lowest quintile, although the relationship was modified greatly by the BMI; for example, women with a BMI of less than 23 did not show an increased risk, but for those with a BMI higher than 23, the relative risk was doubled. These data suggest that the presence of insulin resistance plays an important role in modifying the effect of dietary glycemic load on the risk of CHD. These findings were not confirmed in the Zutphen Elderly Study, which included an elderly cohort [160]. Glycemic load was found to be associated with fasting triglyceride levels in a cross-sectional study of 280 postmenopausal American women [161]. For the lowest and highest quintiles of dietary glycemic load, the mean triglyceride levels were 0.98 and 1.75 mmol/l, respectively. A consistent and very interesting observation is that the dietary GI is a good predictor of HDL levels in the normal population; thus, high-GI diets are associated with lower HDL cholesterol levels [138,162,163].

3.4.3 Conclusion

No clear evidence from dietary trials links sugar consumption and CHD; however, the American Heart Association [158] recommends avoiding a high sugar intake. They state that sugar has no nutritional value other than to provide calories. Long-term, randomized clinical studies are needed to assess the existence of a threshold at which sucrose and fructose content cause abnormalities in lipids. Because the consumption of sugars appears to be increasing, such studies are of utmost importance for constructing therapeutic diets and sound public health recommendations to prevent CVD.

3.5 Sugar Consumption and Micronutrient Intake

Among the main reasons commonly stated for discouraging the consumption of a diet with a high sugar intake is that sugars are empty calories [164]. This stems from the concept that if sugars make up a high proportion of the energy intake they will promote satiety, thus reducing the intake of more nutritious
foods and leading to an inadequate intake of fiber and micronutrients. An excessive consumption of empty calories has been claimed to cause deficiency syndromes [165], although such disorders have never been identified; nevertheless, it is obvious that any diet characterized by an extreme composition consumed for extended periods may cause insufficient intakes of certain micronutrients. The pivotal question is whether a diet with a high, but realistic, sucrose content causes an inadequate intake of micronutrients. Theoretically, a high sucrose intake could either increase or reduce the intake of micronutrients. If the excessive sugar intake is used to facilitate the consumption of oatmeal or corn flakes, it would be accompanied by ample amounts of fiber, minerals, and micronutrients and hence increase the nutrient density of the diet. In contrast, if the same amount of sugar is solely used to sweeten tea and coffee or is supplied by soft drinks, it will certainly reduce the nutrient density of the diet. As sugars may be supplied by several different sources it is not possible to predict the outcome of nutrient density from theoretical assumptions.

A number of dietary surveys have compared intakes of minerals, fiber, and micronutrients in groups of subjects with different intakes of sugars. Subjects with the highest absolute intake of sugars (g/d) are those with the highest energy requirements, and they are well supplied with all micronutrients because they also consume large amounts of all other nutrients [165]; consequently, to make comparisons between groups their intakes should be expressed as sugar E%. Comparing low and high sugar consumers (10 vs. 20 E% from added sugars), those with high sugar intakes consumed less fat and protein (absolute values), but also less vitamin D. By contrast, no significant differences were found in intakes of fiber, calcium, phosphorus, iron, retinol, carotene, riboflavin, vitamin C, or folic acid [165]. Special attention should be paid to thiamin, because high sugar consumption increases requirements for thiamin. Although it is appropriate to express intakes of most micronutrients in absolute figures because requirements do not increase with increasing physical activity, looking at the intake in relation to total energy intake or carbohydrate intake should assess the sufficiency of thiamin intake. In the above-mentioned study, however, thiamin (mg/MJ energy) did not differ between high and low sugar consumers [165].

Despite adequate average intakes, a skewed distribution may conceal a substantially higher proportion of subjects with insufficient thiamin intakes. Rugg-Gunn et al. [165], however, showed that as many subjects among high-sucrose as among low-sucrose consumers met the recommended daily allowances (RDAs) for calcium, iron, thiamin, riboflavin, vitamin C, or retinol, although this was not true for protein. In the COMA report on dietary sugars [166], the intakes of nutrients among 217 adults were compared in the highest, middle, and lowest intakes of nonmilk extrinsic sugars. Here it was also found that subjects in the upper tertile of sugar intake ingested similar amounts of magnesium, zinc, iron, thiamin, calcium, and vitamin C compared to subjects
in the lowest tertile, except for women who had slightly lower intakes of zinc and iron. A further subgroup analysis suggested that women in the lowest tertile of energy intake (794 to 1777 kcal/d) had intakes of magnesium, zinc, and iron below RDAs, and on this low energy intake those with the highest sugar intake had the lowest intakes of the aforementioned nutrients [166]. Their very low levels of energy intakes are, however, insufficient to support life and can only be explained by substantial underreporting, which casts doubts upon the conclusions. Lewis et al. [167] compared nutrient intakes in moderate vs. high consumers of added sugars. If stratified according to g sugar per kg body weight, high sugar consumers had generally higher intakes of vitamins A, C, B6, B12, thiamin, riboflavin, niacin, iron, calcium, and magnesium [167]; however, if sugar intakes were expressed in E%, then an obverse pattern was found. The mean daily intake of most micronutrients exceeded 100% RDAs among the high sugar consumers, but intakes lower than 100% of RDAs were seen for vitamin B6, calcium, and phosphorus in both low and high sugar consumers. Unfortunately, the proportion of subjects below RDAs was not reported [167].

Bolton-Smith [168] compared mean daily nutrient intakes for women consuming <10% energy and 10 to 30% energy from extrinsic sugar and found a lower intake of fat, fiber, vitamin C, vitamin E, and β-carotene, but a similar vitamin A intake, in those women eating a diet with more than 10% sugar energy as compared to those eating a diet with less than 10% sugar energy. The lower intakes of vitamin E and β-carotene were likely to be of minor biological relevance, because vitamin E requirements are lower with a lower intake of polyunsaturated fat. The lower intakes of fiber and vitamin C may be more important. Other studies, however, have found higher intakes of vitamin C and similar intakes of fiber among children having high intakes of sugars as compared to children having low sugar intakes [169].

According to the 1985 National Food Survey [170], subjects from the highest quartile of fat energy (mean, 50 E%) had significantly lower intakes of sugar (5 E%); fiber; vitamins B1, B6, and C; iron; and iodine than subjects from the other three quartiles. Similarly, those from the highest quartile of sugar intake (16 E%) had lower intakes of fat; fiber; vitamins A, D, E, B1, and C; iron; and iodine [170].

In the more recent 6-month CARMEN trial on simple vs. complex carbohydrates, micronutrient and vitamin sufficiency was studied in a subgroup of 46 Danish males and females by self-reported intake data [171]. When comparing diet groups, a lower intake of zinc in men and of vitamin B12 in both men and women was found on the fat-reduced, simple sugar diet compared to the habitual, normal-fat diet. Intakes of these two micronutrients were, however, adequate compared to the Nordic Nutrient Recommendations [2]. No other diet differences were seen. These findings are in line with recent reviews suggesting that nutrient inadequacies on high-sugar diets occur mainly
in vulnerable groups with low energy intakes, mostly women and children [168]. In the healthy adult population, nutrient adequacy can apparently be achieved across a wide range of dietary sugar intakes (4 to 20 E%) [172].

Nutrient density was recently examined in the diets of 983 Danish children, ages 4 to 14 years, with a focus on 10 vitamins and 9 minerals [173]. For all nutrients, the nutrient density declined with increasing energy contribution from added sugar. The only exception was vitamin C, probably due to the intake of sugar-sweetened fruit products. The dilution effect was not equally important for all micronutrients. Vitamins A, B6, B12, and C; thiamin; riboflavin; niacin; and phosphorous are so adequately present in the Danish diet that even a high intake of added sugar does not jeopardize their intake. For folate, calcium, magnesium, and zinc, however, the recommended level is only reached if the sugar E% is appropriately low. Dietary fiber and the remaining micronutrients — vitamins D and E, iron, selenium, and potassium — barely reach the recommended levels even at low sugar intakes in the children’s diet.

In conclusion, the intake of most nutrients is positively correlated with total energy intake; therefore, the lower the energy intake, the higher the risk of micronutrient inadequacies. Sugars may dilute nutrient intake, especially added sugars and liquid sugar in the form of soft drinks. Groups with a low energy intake (elderly, women, and children) are at greatest risk of micronutrient deficiency.

### 3.6 Sugars in Other Conditions

In 2003, the Danish Nutrition Council published a report summarizing the scientific literature regarding sugar intake and health [174], including dental caries, cancer, bone mineralization, infections, allergy, intolerance, children’s growth, behavior, learning, and carbohydrate craving.

Regarding caries, a relationship between a high intake as well as a high frequency of sugar intake and the incidence of dental decay was found; however, regular tooth brushing with fluoride toothpaste seems to compensate for the effects of a moderate intake of added sugars.

Studies in rats have suggested an association between the consumption of sugars and cancer in the gut [175]. In humans, in general, no associations between a high consumption of added sugars and cancer was found [174]; however, in a metaanalysis of 21 population studies, 9 studies showed a positive association between colon cancer and a high sugar intake [174]. Still, 12 studies found no such association [174], so further studies are needed to support this finding.

With regard to bone mineralization, infections, allergy, intolerance, children’s growth, behavior, learning, and carbohydrate craving, no associations with a high sugar intake could be documented from the available literature [174].

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REFERENCES


Cell-Wall Polysaccharides: Structural, Chemical, and Analytical Aspects

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4.1 INTRODUCTION

The cell walls of plants consist mainly of polysaccharides, protein, and lignin. These biopolymers are organized, together with small amounts of other components such as acetyl groups and phenolic substituents, in complex three-dimensional structures that are neither uniform nor completely described in different plants or plant fractions [1–3]. A schematic representation of chemical associations between polymers in lignified cell walls is shown in Figure 4.1 [4]. Cell-wall polysaccharides in foods are a complex group of components differing widely in physical properties and nutritional effects [5]. The recent upsurge of interest in polysaccharides has resulted from the development of more sophisticated and accurate methods of analysis and the realization that cell-wall polysaccharides not only are structural and energy-yielding compounds but can also regulate the utilization of other dietary components in the food [2] and affect animal and human health [6].

Information about the composition and organization of plant cell walls is fundamental for our understanding of the molecular mechanisms of cell-wall polysaccharides that are of technological and nutritional importance. In this chapter, some aspects of the plant cell wall are presented, as well as important procedures for the extraction, purification, and structural elucidation of cell-wall polysaccharides. Major polysaccharide structures in cereals, fruits, and vegetables are also presented, but hydrocolloids, especially from legumes and algae, are discussed in Chapter 6 of this book. In the final section of this chapter, various analytical methods for plant carbohydrates and dietary fiber are presented and discussed.

4.2 PLANT CELL WALLS

A relatively rigid primary wall that develops around the protoplast, exterior to the plasmalemma, is believed to be an integral part of the evolution of plants for life on land [7]. Multicellularity allows variation in the thickness, chemical composition, and spatial distribution of cell walls within tissues [8]. The lignification and resultant increase in hydrophobicity and strength of secondary cell walls are also believed to be an important step in the evolution of land plants [9].

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The primary cell wall, laid down while the cells are dividing and expanding, constitutes the outermost part of the wall. It is built up with a base structure of cellulose microfibrils and matrix polysaccharides, and in some cell types it is the only wall formed. Contiguous cells are separated by a middle lamella (about 0.1 to 0.2 \( \mu m \)) of amorphous pectins and associated calcium ions. Removal of calcium ions usually leads to cell separation [1].

Foods are often selected to contain low amounts of lignified secondary cell walls. This wall is laid down inside the primary wall after cell expansion is completed. It is often comprised of several layers that are differentiated ultrastructurally by their different orientation of cellulose microfibrils, which are stabilized by hydrogen bonding both within and between the glucan chains [10]. The secondary cell walls are thicker (1 to 3 \( \mu m \)) than the primary wall and provide structural strength through their ability to resist tension and compressive forces. Lignification is initiated in the middle lamella and primary wall and proceeds throughout the secondary wall as the cells age. In lignified cell walls, the concentration of lignin is highest in the middle lamella and primary cell wall, but the total amount is highest in the secondary cell wall due to its much greater thickness.

The tertiary wall is a thin membranous layer on the lumen side of the secondary cell wall. This layer sometimes remains as a thin strip after microbial degradation. In some woody cells, a special modification of the tertiary wall,
named the Warty layer, has been observed. This amorphous layer appears to arise from the condensation of the protoplast, together with lignin precursors. In mature barley straw, this layer has been reported to be indigestible and to protect the secondary cell walls from microbial degradation from the lumen side [11].

Several types of tissue with different walls, from both a compositional and structural point of view, are present in plants [7]. The outer tangential walls of epidermis cells become thickened, lignified, and completely covered with a cuticle and waxy layer. Mesophyll contains thin-walled, chlorenchymatous cells and constitutes the main volume of tissue in all leaves but only a small part of the volume of straw. Mesophyll cell walls are not lignified and are generally regarded as a highly digestible cell type [12]. Nonchlorenchymatous parenchyma cells are often quite large cells that may be lignified to a variable degree. The parenchymatous tissues are common in seeds, fruits, and vegetables.

The collenchyma cells show a pronounced thickening of the primary walls that occurs across the corners in an annular shape. This thickened wall does not lignify. Fiber cells in sclerenchyma are long and narrow. They develop a secondary wall and become lignified with maturity. Several types of cells build up the vascular tissue in a plant. Most of them are thick walled, heavily lignified, and undigestible.

### 4.3 EXTRACTION OF POLYSACCHARIDES

Several procedures are available for the extraction of polysaccharides from plant material, but no general method exists. This is understandable, considering the structural complexity and natural variation of polysaccharides in plants which depend on, for example, the species, organs, stage of development, and conditions of growth.

#### 4.3.1 SAMPLE PREPARATION

The sample preparation preceding the various extraction steps in the isolation procedure is of vital importance, as the influence of native enzymatic activity must be minimized and microbial degradation avoided. Enzymes bound to the cell wall may become activated and cause polysaccharide degradation due to inevitable tissue damage during sample preparation; therefore, suitable precautions include the removal of water as soon as possible from freshly harvested plants, preferably by freeze-drying or homogenization in aqueous ethanol followed by drying at about 40°C. This is because drying at elevated temperatures, recommended only for separate determination of the dry matter content, may cause hydrolytic degradation of polysaccharides [13] or Maillard reactions [14].
4.3.2 EXTRACTION CONDITIONS

When cell-wall polysaccharides are to be extracted, the choice of procedure is important for the result and should, therefore, be planned in relation to the scope of the investigation. Polysaccharides (e.g., arabinoxylans, arabinogalactans, mixed-linkage β-glucans, and pectins, including associated neutral polysaccharides) are found as soluble and insoluble components in many plant materials. There is generally no sharp distinction between soluble (or extractable) and insoluble fractions for several polysaccharides; the ratio between them is simply dependent on conditions used (e.g., physical pretreatment, enzymatic treatment, temperature, time) during the solubilization procedure. The yield of these components can, therefore, vary considerably with the extraction conditions. Studies [15,16] have shown that, in addition to the yield, the composition of soluble polysaccharides is also very dependent on the extraction conditions.

4.3.3 REMOVAL OF NON-CELL-WALL COMPONENTS

After the initial sample handling, non-cell-wall components such as lipids, proteins, phenols, and free sugars can be removed by different extractions. The isolation of cell-wall polysaccharides often begins with the preparation of an alcohol-insoluble residue by dispersing finely ground or homogenized plant material in hot, 80 to 90% (v/v) aqueous ethanol and washing the residue with absolute ethanol and ether or acetone. This treatment removes low-molecular-weight carbohydrates and other soluble components and also minimizes the activity of cell-wall-degrading enzymes. Next, non-cell-wall protein may be extracted with a mixture of phenol–acetic acid–water that dissolves intracellular protein efficiently but polysaccharides poorly [17]. When present, starch can be removed by dissolution in 90% (v/v) aqueous dimethyl sulfoxide [18]. An alternative, effective system for enzymic removal of starch is to use thermostable α-amylase at 96°C in acetate buffer followed by incubation with amyloglucosidase at 60°C [19,20]. Proteins can also be removed by enzymatic hydrolysis.

4.3.4 EXTRACTION OF CELL-WALL POLYSACCHARIDES

Preparation of aqueous extracts containing polysaccharides is a common initial step in the isolation of polysaccharides from plants. Thus, pectic substances are extracted with aqueous solutions of chelating agents, such as ethyldiamine tetraacetic acid (EDTA) or ammonium oxalate [18]. The action of the chelating agents results from their ability to combine with calcium (Ca) and magnesium (Mg) ions, which are known to complex with pectins. Other studies [21,22] have shown that the extraction of pectins with 1,2-cyclohexane diaminetetraacetate (CDTA) is sometimes preferable because
degradation of pectins by β-elimination [23,24] becomes less significant. The CDTA-insoluble pectic polysaccharides are usually highly branched and probably ester cross-linked with the wall matrix. These insoluble polysaccharides may be extracted with dilute sodium carbonate or sodium hydroxide, if certain precautions are taken — low temperature, nitrogen atmosphere, and addition of reducing agents — in order to minimize β-elimination and other reactions modifying the structure.

Other noncellulosic polysaccharides (e.g., arabinoxylans, xyloglucans, glucomannans) are preferentially extracted using aqueous solutions of alkali containing sodium borohydride. The borohydride converts the reducing end groups of the polysaccharides to a hydroxymethyl group, and this decreases the incidence of alkaline degradation [18]. If lignin is present in the plant tissue, delignification with, for example, sodium chlorite–acetic acid may be necessary before the polysaccharides are extracted with alkali [25]. During the lignin removal step, however, some proteins and cell-wall polysaccharides may be lost due to solubilization and degradation reactions. Cellulose resists extraction with alkali but has been solubilized by N-methylmorpholine N-oxide [26].

When enzymic methods are used in combination with traditional chemical extraction methods, this can provide increased knowledge of the cell-wall architecture, although the pure well-defined enzymes that are needed are not generally available [27–29]. Endopolygalacturonases, endoglucanases, and endoglucuronoxylanase are examples of enzymes that have been used for specific degradation of the cell-wall matrix. Other important components of the cell-wall matrix are glycoproteins and proteoglycans [30]. Degradative conditions are required for the solubilization of these components, such as treatment with alkali, chlorite–acetic acid, or cell-wall-degrading enzymes.

4.4 PURIFICATION OF POLYSACCHARIDES

The polysaccharide extracts often require further purification by various fractionation techniques to give sufficient homogeneity before a meaningful structural analysis of the polysaccharide can be performed. The purification procedures vary so much because of the wide variations in chemical and physical properties of polysaccharides. These procedures can be based on differences in solubility, molecular weight, or chromatographic properties.

4.4.1 Methods Based on Solubility Differences

The solubility of polysaccharides in aqueous solution changes with the addition of different agents. Alcohol or iodine, copper, barium, and quaternary ammonium salts have been used to decrease solubility and cause precipitation [1]. Some examples of these techniques are given below, but they constitute a very
minor part of all reported procedures. Precipitation of polysaccharides in 90% ethanol has been used to obtain fractions of arabinoxylans in rye [31] and wheat flour [32] for further study of the structural characteristics of these polysaccharides. Graded precipitation with ethanol was used to isolate arabinoxylan fractions from wheat flour [33], and arabinoxylans in rye have been separated using graded precipitation with ammonium sulfate [34]. Branched and linear hemicellulosic polysaccharides in Leguminosae and Graminae were separated by the difference in their ability to complex with iodine [35]. Glucomannans and mannans have been precipitated with barium hydroxide, probably due to reaction with 2,3-cis-hydroxyl groups in the mannose residues [36]. Separation of acidic (pectic) polysaccharides from neutral ones can be performed by using quaternary ammonium salts [37].

4.4.2 Methods Based on Differences in Molecular Weight

Ultracentrifugation, membrane ultrafiltration, and gel filtration are other purification procedures that are based on differences mainly in the molecular weight and conformation of the polysaccharides. These and other techniques, such as light scattering, have also been used for determination of molecular weights [38]. Size-exclusion chromatography coupled with online, low-angle laser light scattering is a valuable analytical tool for the characterization of polysaccharides [39]. Gel filtration is a common fractionation technique, and a number of applications have been described. Water-soluble, mixed-linkage β-glucans in oats were reported to have high molecular weights — about 1,000,000, as determined by this technique [40]. A water-soluble arabinoxylan and an arabinogalactan peptide, both present in wheat flour, have been partially separated by gel filtration on Sepharose 4B (Figure 4.2) due to the large difference in molecular weight between them [41]. Complete separation of these polysaccharides was achieved after treatment with saturated ammonium sulfate, because the arabinoxylan fraction was precipitated, whereas the arabinogalactan remained soluble. High-performance, size-exclusion chromatography on the Ultropac TSK-GEL G 5000 PW and 6000 PW was used to separate β-glucans into well-defined fractions [42] from a polydisperse oat aleurone sample.

4.4.3 Methods Based on Chromatography

Purification by adsorption, ion-exchange, and partition chromatographic methods has gained increased importance. A few of the many applications [1] are outlined below. Diethylaminoethyl (DEAE)–cellulose is often used with slightly acidic phosphate buffers as eluents [43,44]. Fractionation of pectins can be achieved based on their degree of esterification, and low-esterified
pectins are generally eluted at an increased ionic strength of the buffer [45]. Strong acidic pectic polysaccharides can be initially separated from weakly acidic ones using Sephacel in chloride form [46]. DEAE-Sepharose CL-6B has been used in the fractionation of carrot polysaccharides [47] as well as xyloglucans as borate complexes [48]. Quaternary aminoethyl (QAE)–Sephadex was efficient in the fractionation of papaya pectin polysaccharides [49]. Sometimes low and varying recoveries have been obtained with these methods, which is a drawback. The reason for this may be interaction between polysaccharides or polysaccharide complexes in the sample and the column material or the eluent used. Glycoproteins have also been purified by affinity chromatography using the ability of their oligosaccharide part to interact with lectins, which are proteins capable of binding to a particular sugar residue with a designated anomic configuration [50,51].

4.4.4 Effects on Structure

It should be noted that the properties and structures of polysaccharides might be changed during extractions, fractionations, and other treatments of the plant material; for example, when present, ester groups will be removed by alkaline treatment, and degradation of pectic polysaccharides can occur by β-elimination. Other problems may include depolymerization when treated with shear forces and that only a small fraction of the polysaccharide is isolated and that this fraction may not be representative of the polysaccharide structure present in the starting material. For a more detailed discussion on the subject of extraction and purification of plant polysaccharides, see the various reviews that have been published [1,18,52,53].
4.5 STRUCTURAL DETERMINATION OF POLYSACCHARIDES

When the polysaccharide has been purified, it can be subjected to structural analysis by many different methods [54,55]. In order to determine the primary structure of the polysaccharide in detail, several characteristics must be elucidated.

4.5.1 MONOSACCHARIDE COMPOSITION

The composition of the monosaccharides (Figure 4.3) that are present as the building blocks of the polymer [56] is initially determined. The compositional analysis of carbohydrate polymers is often based on methods involving acid hydrolysis to release constituent sugars, followed by gas–liquid chromatography (GLC) or by high-performance liquid chromatography (HPLC) as the end determination step. The acid hydrolysis is usually performed by a sequential procedure, and the conditions are critical in order to obtain complete hydrolysis of the polysaccharides with as little sugar degradation as possible [52,57]. The neutral monosaccharides released may conveniently be quantified as the corresponding alditol acetates by GLC [58,59], but other types of derivatives, such as aldononitrile acetates [60] and O-methyloxime acetates [61], have also been used. Analysis of monosaccharides by HPLC is still not as common as by GLC. An advantage of HPLC compared to GLC is that preparation of derivatives is not required — for example, in the determination of monosaccharides [62–64] with amperometric detection, which is gaining in importance. Detailed discussions regarding the advantages and disadvantages of using HPLC [65–67] and GLC [68,69] methods have been published.

The acidic sugar constituents of polysaccharides, the so-called uronic acids, are more difficult to quantify than those of neutral sugar constituents. The release of these acids as monomers is complicated by the high stability of the glycosyl uronic acid linkages under acidic conditions, resulting in incomplete hydrolysis. Also, once released, the uronic acids are under acidic conditions and more rapidly degraded to noncarbohydrate products than neutral sugars. Colorimetric methods have been developed for the measurement of uronic acids in plant materials [70–72]. A disadvantage with colorimetry is the sensitivity to the reaction conditions as well as possible interference from other components in the reaction mixture (e.g., neutral sugars and proteins). A stoichiometric procedure for the analysis of the content of uronic acids by decarboxylation in hydroiodic acid with subsequent measurement of the carbon dioxide evolved as HCO₃⁻ has been developed [19,73]. This method avoids the problems of incomplete hydrolysis of uronic acids but, like colorimetric methods, only measures the sum of uronic acids and not the individual sugar constituents. Specific analysis of uronic acids has been performed by GLC as trimethylsilyl ethers of methyl glycosides [74,75] obtained on methanolysis of the parent
A GLC method for the simultaneous determination of uronic acids in hydrolysates (as N-hexylaldonarnide derivatives) and of aldoses (as alditol acetates) has also been reported [76]. Analysis of individual uronic acids by HPLC without previous derivatization using pulsed amperometric detection was reported [77].

4.5.2 Determination of Absolute Configuration

The configuration of sugar residues present in a polymer isolated from plants is often assumed, as rhamnose, fucose, and arabinose are usually L, while polysaccharide. A GLC method for the simultaneous determination of uronic acids in hydrolysates (as N-hexylaldonarnide derivatives) and of aldoses (as alditol acetates) has also been reported [76]. Analysis of individual uronic acids by HPLC without previous derivatization using pulsed amperometric detection was reported [77].
mannose, galactose, glucose, glucuronic acid, and galacturonic acid are D. The absolute configurations, however, have to be determined when an entire characterization is needed. Optical rotation measurements and specific oxidation of sugars with enzymes have been used earlier, but today the configuration can conveniently be established by GLC methods [78–80]. These are based on the preparation of the corresponding butyl- or octylglycosides using enantiomeric (−)-2-butanol or (+)-2-octanol, followed by comparison of GLC retention times for suitable derivatives (silylthers or acetates). A related approach separates sugars as derivatives on columns coated with stationary chiral phases [81].

4.5.3 Determination of Glycosyl Linkages and Ring Forms

More advanced techniques are often required for determination of these characteristics, such as methylation analysis combined with GLC–mass spectrometry (MS) [82–84]. This is based on the conversion of all free hydroxyl groups in the polysaccharide to methyl ethers by reaction with methyl iodide in alkaline solution (i.e., per-O-methylation is accomplished). The individual sugar residues are then released by acid hydrolysis, reduced, acetylated, and analyzed by GLC as partially methylated alditol acetates, often in combination with mass spectrometry. The glycosyl linkage type can be deduced from the pattern of MS fragmentation of these derivatives. The ring forms of sugar residues (i.e., furanose or pyranose) that occupy a terminal position in the polymer are established by the pattern of O-methyl substitution resulting from methylation analysis, but some interchain sugar residues (4-linked hexopyranosides and 5-linked hexofuranosides) will produce identical fragmentation patterns by MS. The differentiation of these relies on the chromatographic separation or incorporation of deuterium during the borohydride reduction step preceding acetylation. As an alternative, other methods such as nuclear magnetic resonance (NMR) spectroscopy [85] or a more complex analysis method [86] (see Section 4.5.6) can be used to verify the ring form. Uronic-acid-containing polymers, such as rhamnogalacturonans in pectins, are sensitive to alkaline conditions which might fragment the polymer chain by β-elimination, particularly for 4-O-substituted uronic acids. Also, as discussed above, the uronic acids are quite resistant to acid hydrolysis, and incomplete hydrolysis results in the formation of aldobiuronic acids that escape analysis by the method. One way to circumvent this is to activate the carboxyl groups by carbodiimide esterification followed by reduction to hydroxy methyl groups [87,88]. As an alternative, the carboxyl groups can be derivatized and then reduced with lithium aluminum hydride or deuteride [89].

In an alternative method of structural determination [90] the per-O-methylated polysaccharide can be subjected to reductive cleavage using ethylsilane in the presence of a catalyst. The resulting partially methylated anhydroalditols
can then be readily characterized by GLC–MS of their acetates or by $^1$H–NMR spectroscopy of their benzoates [91]. This NMR method has the advantage that, in addition to the glycosyl linkage and ring form, the identity of the sugar residue is also established.

### 4.5.4 Determination of Anomeric Configuration

When specific enzymes, carbohydrases, are available their hydrolytic action on the polysaccharide may provide information about the type of linkages present [28]. Although optical rotation measurements can indicate the type of anomeric linkage for homopolysaccharides (e.g., amylose), it is becoming obsolete for this purpose. The most suitable methods for establishing anomeric configurations (α or β) as well as whether the sugar residues exist in the furanose or pyranose ring form are $^1$H–NMR [85] and $^{13}$C–NMR [92]. The values obtained for the chemical shift and the coupling constants of anomeric protons make it possible to assign anomeric configurations of both native and derivatized polysaccharides. Several different high-resolution NMR techniques can be used for structural investigation and, among these, basic one- and two-dimensional methods are summarized in a textbook on NMR [93].

### 4.5.5 Investigation of the Sequence of Glycosyl Residues

Plant cell-wall polysaccharides, unlike bacterial polysaccharides, often lack true repeating units. NMR is an important tool for investigation of the fine structure of polysaccharides. Arabinobiosans and many arabinobiosan fragments formed by enzymatic degradation have been characterized by $^1$H–NMR to elucidate the fine structure [94,95]. The natural variations in the content of structural elements of water-extractable arabinobioans in wheat flour were determined using a procedure based on sugar analysis and $^1$H–NMR analysis of extracts [31,32]. The anomeric region of a $^1$H–NMR spectrum of a fraction containing arabinobioans is shown in Figure 4.4 with interpretation of the main signals [96]. The number of anomeric signals reflects the number of differently linked sugar residues, the molar proportion of which can be calculated from the corresponding integrals. Various methods based on $^1$H–NMR and $^{13}$C–NMR [97,98], affinity on DEAE–Sephacel [99], or calcium activity in solutions of calcium pectinate [100] have also been devised to differentiate between a random or blockwise distribution of ester or carboxylic groups in the main polysaccharide chain. The fine structure of oat mixed-linkage β-glucans has been studied by $^{13}$C–NMR [101–103]. One approach in the investigation of the glycosyl sequence in a polysaccharide is based on degradation by acid or enzymes [28,104] followed by methylation analysis of the oligomeric fragments obtained. The structural information gained is then combined in order to define the structure of the parent polysaccharide.
Mass spectrometric techniques are valuable in the studies of oligomeric fragments of polysaccharides, and several techniques have been reviewed [105]. Matrix-assisted laser desorption/ionization in combination with time-of-flight mass spectrometry is a very useful method for the determination of the molecular mass of underivatized oligosaccharides [106,107]. The molecular weight of glucans has been accurately measured up to about 7000 Da in an oligomeric dextran mixture [108]. Fast atom bombardment mass spectrometry (FAB–MS) can be used to determine linkage positions between monosaccharide residues [109]. The chemical procedure involves cleavage of vicinal hydroxyl groups in sugar residues by periodate, followed by reduction, permethylation, and FAB–MS [110]. The sequence has been determined for permethylated oligosaccharides with eight to nine sugar residues using GLC–MS [111].

**4.5.6 Procedure for Complete Determination of Polysaccharide Structure**

In addition to the characteristics discussed in the previous sections, the identity and points of attachment for any noncarbohydrate moieties present (and, if
appropriate, stereochemistry) must also be determined to achieve a complete characterization of the polysaccharide primary structure. Such a procedure has been developed by McNeil et al. [86] and is comprised of the following steps:

1. The polysaccharide is purified and the composition of glycosyl residues determined. The anomeric configuration and noncarbohydrate substituents when present are established, if possible, by NMR of the intact polysaccharide.

2. Uronic acid residues are prereduced, if appropriate or possible, and the polysaccharide is methylated. An aliquot of the methylated sample is used for analysis of its glycosyl linkage composition and the ring form. Carboxyl groups of uronic acid residues may be reduced at this stage.

3. The methylated polysaccharide is partially hydrolyzed under optimized conditions and the resulting oligomers reduced and ethylated.

4. The mixture of peralkylated oligosaccharide alditols is fractionated by HPLC and the alditols detected by refractive index or HPLC–MS. Isolated peralkylated di-, tri-, or tetrasaccharide alditols may be analyzed by GLC–MS and higher alditols by direct probe MS or FAB–MS. The anomeric configurations of the glycosyl linkages of the isolated alditols are determined by $^1$H–NMR.

5. The fractionated oligosaccharide alditols are fully hydrolyzed, reduced, and acetylated. The resulting partially alkylated and acetylated alditols are characterized by GC and GC–MS. The glycosyl sequence of the fractionated peralkylated oligosaccharide alditols is deduced and the ring form of the glycosyl residues determined.

6. The primary structure of the polysaccharide is determined from its glycosyl composition, the structure of the oligosaccharides derived from it, and the content of any labile noncarbohydrate component.

7. The molecular weight distribution of extracted polysaccharides is determined, generally by size-exclusion chromatography in combination with different detectors. Purified polysaccharides can be analyzed with high-performance size-exclusion chromatography combined with refractive index and multiple-angle laser light scattering (HPSEC-RI-MALLS) [112]. Certain polysaccharides, such as cereal β-glucan, can be analyzed in mixtures by using SEC in combination with specific fluorescence detection [113]. This detection is based on specific binding of Calcofluor to the β-glucan molecules.

### 4.6 POLYSACCHARIDE STRUCTURES IN CEREALS

Parenchymatous and lignified tissues predominate in cereals [1]. The cell walls in these tissues consist of a reinforced, multicomponent matrix of cross-linked
Cellulose
\(-4\)-\(\beta\)-D-Glc\(\rightarrow\)\(\beta\)-D-Glc\(\rightarrow\)\(\beta\)-D-Glc\(\rightarrow\)\(\beta\)-D-Glc

Mixed-linkage \(\beta\)-glucan
\(-4\)-\(\beta\)-D-Glc\(\rightarrow\)\(\beta\)-D-Glc\(\rightarrow\)\(\beta\)-D-Glc\(\rightarrow\)\(\beta\)-D-Glc\(\rightarrow\)\(\beta\)-D-Glc\(\rightarrow\)\(\beta\)-D-Glc\(\rightarrow\)\(\beta\)-D-Glc\(\rightarrow\)\(\beta\)-D-Glc\(\rightarrow\)
\(n=1\) or \(2\) (~90%); \(n=3-12\) (~10%)

FIGURE 4.5 Structural features of cellulose and mixed-linkage \((1\rightarrow3),(1\rightarrow4)\)-\(\beta\)-D-glucans.

polymers in which a network of cellulose microfibrils is embedded [114]. Major matrix polysaccharides are mixed-linkage \((1\rightarrow3),(1\rightarrow4)\)-\(\beta\)-D-glucans (mixed-linkage \(\beta\)-glucans), arabinoxylans, acidic xylan, and cellulose. Although polysaccharides are the major components of the wall matrix, structural proteins, including glycine-rich proteins, threonine-rich glycoproteins, and hydroxyproline-rich glycoproteins, may form a second network within the matrix phase [115]. Lignin and phenolic acids may be covalently linked to the matrix polysaccharides as discussed previously (see Figure 4.1).

Cellulose (Figure 4.5) is a homopolysaccharide composed of 4-linked \(\beta\)-D-glucopyranosyl residues with wide molecular weight distributions and, from a conformational point of view, anhydrocellobiose as the repeating unit [116]. The extended molecule forms a flat ribbon, which is further stiffened by intra- and intermolecular hydrogen bonds. The hydrogen bonds produce a regular crystalline arrangement of the glucan chain, resulting in distinct x-ray diffraction patterns and low solubility. Native cellulose is, however, composed of both highly ordered crystalline and non-crystalline regions.

Mixed-linkage \(\beta\)-glucan is present as an important component of the endosperm cell walls of cereals, especially in barley and oats [117]. Isolated fractions of the linear homopolysaccharide are composed of about 30% 3-linked and 70% 4-linked \(\beta\)-D-glucopyranosyl residues, but they generally also contain smaller amounts of protein, arabinose, and xylose residues [103,115]. About 90% of the 4-linked residues occur in groups of two or three residues, separated by single \((1\rightarrow3)\) linkages (Figure 4.5) [118]. The resultant polysaccharide is therefore mainly composed of 3-linked cellotriosyl and cellotetraosyl units. Isolated fractions also contain longer sequences of 4-linked residues that may be of importance for the attachment of the polysaccharide in the wall matrix. The distribution of cellotriosyl and cellotetraosyl blocks in the polysaccharide chain and the incorporation of longer sequences of 4-linked residues in the structure remain to be understood. Recent studies, however, indicate that the cellotriosyl blocks are present in a random order in the polysaccharide backbone [119]. The molecular weight of isolated mixed-linkage \(\beta\)-glucan fractions has been reported to vary between 20,000 and 40,000,000 g/mol [114], but the values obtained depend very much on the isolation procedure of the
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polysaccharide fraction and the method used for the estimation. In recent studies, the average molecular weights of extracted mixed β-glucan from barley and oats were around 2,000,000 g/mol and significantly lower in extracts from rye and wheat [113]. Extracts from oats, oat bran, and oat bran concentrate had very similar molecular weights [120].

Nonfibrillar 3-linked β-glucans are found in specialized cell walls and wall layers or deposited as callose in response to wounding, infection, or physical stress [115,121]. In starchy endosperm of several cultivars of barley, small head-like deposits of 3-linked glucans are found on the inner surface of the cell walls [122]. In the subaleurone region, larger deposits can be found sometimes completely embedded in wall material. The total content of 3-linked β-glucan in barley has been reported to be around 1% but is likely to vary considerably among cultivars and cereals [122].

Acidic xylans are generally present in lignified tissues of both monocotyledons and dicotyledons and are often isolated by alkaline extraction [1,29]. They consist of a backbone of 4-linked xylose residues with short side chains of mainly arabinose, glucuronic acid, and 4-O-methyl-glucuronic acid residues [115,123]. Up to 90% of the xylose residues in the backbone may be unbranched. A significant portion (5 to 17%) of the xylose residues may be acetylated, mainly on the hydroxyl groups of C-2 but also on C-3 [124]. The insoluble, bound p-coumaric acid of barley grain occurs mainly in the outer grain layer [125]. It is, however, not known whether this phenolic constituent is chemically linked to the heteroxylan structures.

Arabinoxylans are the other major group of polysaccharides in endosperm cell walls of cereals [114]. These heteropolysaccharides consist predominantly of arabinose and xylose residues and are therefore often referred to as pentosans. Arabinoxylans consist of a main chain of 4-linked D-xylopyranosyl residues that is substituted mainly at the C-3 or the C-2 and C-3 positions of the xylose residues (Figure 4.6). In wheat and rye, only small amounts of C-2-substituted xylose residues are present, but in isolated arabinoxylan fractions of dehusked barley grain 10 to 30% of the xylose residues have been shown to be substituted by arabinose residues in this way [126,127]. A small proportion of oligomeric side chains, consisting of two or more arabinosyl residues or an arabinosyl residue with a terminal xylosyl residue, has also been reported [127]. Arabinoxylans may also carry phenolic acids, such as ferulic acids, as substituents [114].

Our studies on water-soluble arabinoxylans in rye grain showed that two polysaccharide structures were present. The major fraction, arabinoxylan I, was characterized by a main chain of xylose residues, of which about 40% were substituted at the C-3 position by terminal arabinose residues [128]. The other polymeric fraction, arabinoxylan II, was isolated by gel filtration after treatment with xylanase [129]. This structure had a main chain of 4-linked xylose residues, of which about 70% were substituted at both the C-2 and C-3
positions with terminal arabinose residues. No xylose units were solely mono-
substituted. From these studies, it can be concluded that the mono- and dis-
substituted xylose residues could be present in different polymers or in different
regions of the same polymer. It also follows that the fine structure of arabi-
noxylans in rye is less randomly organized than was previously believed
[128,129]. Recent work on water-insoluble arabinoxylans from barley endo-
sperm also indicates that the distributions of arabinosyl substitutents along the
xylan backbone are nonrandom [126,127]. In some regions, substituted xylose
residues are clustered together, often separated by single unsubstituted resi-
dues. Other regions contain relatively few arabinosyl residues and are therefore
susceptible to hydrolysis by xylanases. These substitution patterns will
undoubtedly influence both the chemical and physical properties of the poly-
saccharides and thereby the technological and nutritional properties of cereal
products. Today, structure heterogeneity is a well-known fact for all cereals
studied [130].

Rye arabinoxylans have been classified according to extractability and
structure [130]. There are four distinctively different classes, but their extract-
abilities partly overlap (Figure 4.7). In water extracts, arabinoxylan I (unfilled
part of the bar) dominates. Arabinoxylans containing both mono- and disub-
stituted xylose residues are present in both water and alkali extracts (marked
with stripes). These fractions are not homogenous and can be fractionated into
several subfractions with different degrees of substitution. The third class of
arabinoxylan is very sparsely substituted (acidic xylan) and isolated as a
precipitate after neutralization of alkali extract. Finally, the fourth class of rye
arabinoxylan is a highly branched heteroxylan with a complex structure. About
80% of the xylose residues in the backbone are substituted with terminal
arabinose or glucuronoc acid residues, as well as dimeric and oligomeric chains
with arabinose, xylose, and sometimes galactose residues. Some of the arabi-
nose residues, which always seem to be linked directly to the backbone, carry
feruloyl units at O-5.
Glucomannans consist of a backbone with 4-linked glucopyranosyl and mannopyranosyl residues in which some of the mannose residues may be substituted by single $\alpha\r$-D-galactopyranosyl residues [131]. Unsubstituted glucomannans form insoluble aggregates and can form noncovalent associations with crystalline cellulose in vitro [132]. The presence of about 2% glucomannan in wall preparations from barley aleurone and starchy endosperm has been inferred from the detection of mannose residues in fractions extracted from the walls with concentrated alkali [115]. Mannose residues are generally found in the water-insoluble fraction of wheat [133,134]; however, this polysaccharide has not yet been isolated in a pure form, and its detailed structure remains to be elucidated in cereals.

### 4.7 POLYSACCHARIDES IN FRUITS AND VEGETABLES

Fruits and vegetables consist mainly of growing cells and parenchymatous and lignified tissues [1]. Important polymers in the two former tissues are cellulose, pectic substances with associated polysaccharides, xyloglucans, and some glycoproteins, while in the lignified tissues cellulose, lignin, glucuronoarabinoxylan, and smaller amounts of glycoprotein and pectic substances are important macromolecules. Because distinct differences in macromolecular composition are evident in the two groups of tissue, it is advisable to separate them before chemical analysis. The general structure for cellulose and glucuronoarabinoxylan is the same as that described for cereals.
Pectic polysaccharides are present in the primary cell wall and middle lamella of all seed-bearing plants and have important nutritional and technological properties, mainly because of their ability to form gels [135,136]. They contain galacturonic acid residues and are major components of the primary cell walls of dicotyledons but account for relatively less of the primary walls in the Graminaceae [137]. Pectins contribute to many functions of the cell walls in plant tissues, including physiological aspects related to growth, the determination of cell size and shape, the integrity and rigidity of tissues, ion transport, water holding, and defense mechanisms against infections and wounding [135,138]. The amount and nature of the pectin strongly influence the texture of fruits and vegetables in growing, ripening, and storage and also affect the processing.

Pectic substances are probably the most complex class of plant cell wall polysaccharides. In essence they comprise two families of covalently interlinked acidic polymers, galacturonans, and rhamnogalacturonan (Figure 4.8) [139]. Galacturonans consist of pectin segments containing exclusively 4-linked α-D-galacturonic acid residues in the backbone, such as homogalacturonan, and the substituted xylogalacturonan and rhamnogalacturonan II. Rhamnogalacturonan II is a complex polysaccharide that can be solubilized by endopolygalacturonase and is composed of a backbone of 4-linked α-D-galacturonic acid residues substituted at C-2 or C-3 with aldehydo- and keto-sugar oligosaccharide side chains. These side chains have been reported to contain about 30 different glycosyl residues, of which many are unusual or unique, such as 2-O-methyl-fucose, 2-O-methyl-xylose, apiose, 3-C-carboxy-5-deoxy-xylose (aceric acid), 2-keto-3-deoxymannooctulosonic acid (KDO), and 3-deoxy-lyxo-2-heptulosaric acid (DHA). Rhamnogalacturonans are a family of polysaccharides that has a backbone of an alternating disaccharide containing 4-linked α-D-galacturonic acid and 2-linked α-L-rhamnose residues. About 50% of the rhamnose residues are substituted at C-4, or in some cases at C-3, with oligosaccharides (mainly arabinose and galactose residues) [140]. Arabinans, arabinogalactans, and galactans are reported as being components of rhamnogalacturonan I [141]. Some of the galacturonic acid residues in the pectin are methylesterified [142,143]. Pectins have also been shown to contain feruloyl [144] and acetyl groups [139].

Xyloglucans (amyloids) are generally present in the primary cell walls of higher plants and constitute as much as 20 to 25% of the walls of dicotyledons [145]. Xyloglucans often contain glucose, xylose, and galactose in a molar ratio of about 4:3:1 and sometimes also small amounts of arabinose. They have a backbone of 4-linked β-D-glucopyranosyl residues with side chains of α-L-xlyopyranosyl residues linked to the 6 position of some of the glucose residues and β-D-galactose residues attached to the 2 position of some of the xylose residues. The α-L-fucosyl residues are attached to the 2 position of some of the galactose residues. Heterogeneity of isolated xyloglucan fractions is
due to differences in molecular weight and composition and distribution of the side chains. Because xyloglucans have a backbone of 4-linked β-D-glucopyranosyl residues, they are susceptible to depolymerization with cellulase [146]. The structural characterization of released oligosaccharides has been of tremendous importance for studies on polysaccharide structure and heterogeneity in different plants. It appears that all xyloglucans are composed of a repeating heptasaccharide unit to which variable amounts of galactose, fucose, and possibly arabinose are attached [145]. O-acetyl substitutents have also been detected on xyloglucans, predominantly on the galactosyl residues [147].
As an example, the major repeating unit of pea xyloglucan [148] is presented in Figure 4.9.

The glucan backbone of xyloglucan has an extended, twofold helix conformation similar to cellulose [149]. It appears that the sugar residues in the side chains do not alter the conformation of the glucan backbone. In the primary wall, xyloglucans are highly associated with the cellulose microfibrils, and isolated xyloglucans have been observed to bind to purified cellulose by hydrogen bonds [148,150].

4.8 ANALYTICAL ASPECTS

Many procedures have been developed for the determination of dietary fiber (DF) and its individual components. The advantages and drawbacks of some of these methods are presented below, together with a discussion of some specific methods.

4.8.1 OLD GRAVIMETRIC METHODS

The crude fiber procedure was developed in 1859 by Henneberg and Stohmann [151]. The method uses sequential extraction of plant material with diethyl ether, diluted acid, and diluted alkali. After this treatment, the dry weight of the insoluble residue is taken as the content of crude fiber in the original sample. The method has the serious drawback that variable proportions of lignin and hemicelluloses are lost due to the solubility of these components, mainly in alkali [59]. The merit of the method is mainly its simplicity, but it should not be used for the analysis of dietary fiber in foods.

Van Soest introduced two procedures based on the use of detergents for the quantification of fiber in feed. Extraction of the fiber sample with an acid detergent or a neutral detergent produces the insoluble acid detergent fiber (ADF) [152] and neutral detergent fiber (NDF) [153] residues, respectively. These residues can be further fractionated to obtain separate values, among others, for the contents of cellulose, insoluble hemicelluloses, and lignin [154]. A modification of the NDF method that uses amylase to degrade starch has been adopted by the American Association of Cereal Chemists (AACC) as an official method for determination of dietary fiber in foods (AACC method 32-20). The NDF methods do not measure the soluble polysaccharides, in particular pectic ones, which therefore require determination by another method. A modification of the NDF method [155] uses extraction with urea followed by dialysis to remove nonfiber components from the food sample. Mongeau and Brassard [156,157] combined the NDF procedure with a separate determination of hot-water-soluble fiber, but there is a risk of analyzing some components twice or not at all with this modification [59].
4.8.2 *Enzymatic Gravimetric Methods*

The first enzymatic gravimetric procedures that account for both soluble and insoluble dietary fiber components were developed by Furda (1977), Schweizer and Würsch (1979), and Asp and Johansson (1981) [158–160]. In a modification of these methods, starch and proteins are enzymatically degraded by sequential treatment in aqueous buffers with amylases or proteases. Soluble fiber is then precipitated with four volumes of ethanol. In this way, separate values for the insoluble and soluble part of dietary fiber as well as values for total dietary fiber can be calculated, after correction for the residual protein and ash content of the residues isolated. There is also an option of analyzing the sugar constituents of the polysaccharides by conventional sugar analysis when a detailed chemical composition is needed. A thorough discussion of the importance of various steps in these enzymatic gravimetric methods (e.g., sample preparation, protein degradation, alcohol precipitation, and mineral correction) has been published [59]. One of the enzymatic gravimetric methods has been adopted by the Association of Official Analytical Chemists (AOAC) as a method for determining total dietary fiber [161] and it was later further simplified by the replacement of the initial phosphate buffer treatment [162]. The main advantages with the new buffer are that soluble and insoluble dietary fiber can be analyzed separately, coprecipitation of inorganic material is diminished, one pH adjustment step is removed, and smaller solvent volumes can be used.

4.8.3 *Component Methods*

As a complement to gravimetric procedures, methods based on a more specific determination of the components of dietary fiber have been developed. The gravimetric methods are comparable with the analysis of crude fat and protein in the proximate analysis system, while component analyses of dietary fiber are comparable with the analysis of fatty acids and amino acids, for example. Such component methods may be very useful when physiological, nutritional, and technological properties of dietary fiber are studied.

4.8.3.1 *Methods Based on Colorimetry*

The Southgate procedure for determination of “unavailable carbohydrates” (i.e., the polysaccharides of dietary fiber) was published in 1969 [163]. This procedure measures the monosaccharides in the hydrolysate by colorimetry. Hexoses are quantified by the anthrone method [164], pentoses by the method of Albaum and Umbreit [165], and uronic acids by the carbazole reaction [70]. The major limitations of the procedure are that it relies on colorimetric methods that are only partly specific, and it is technically demanding and rather non-robust [59].
Another enzymatic chemical method for determining the content of non-starch polysaccharides that also uses colorimetry in the end determination step has been published [166] and later modified [77]. In this procedure, starch is removed by extraction with dimethyl sulfoxide (DMSO) and enzymatically degraded in aqueous acetate buffer by treatment with bacterial α-amylase, pancreatin, and pullulanase. Soluble fibers are then precipitated with 80% ethanol and subjected to acid hydrolysis, either alone or together with the insoluble fiber fraction. The monosaccharides released in the hydrolysate are quantified by two colorimetric methods that give values for the neutral and acidic [71] nonstarch polysaccharide contents.

Several procedures have been developed for the analysis of mixed-linkage β-glucan contents, involving enzymatic degradation and measurement of the glucose released by colorimetry [117,167–169]. A fluorescence method for a rapid and direct measurement of β-glucan using Calcoflour as the reagent is also available [170,171].

Cellulose can be estimated by the Southgate procedure or by the Van Soest ADF procedure mentioned above. At present, simple and accurate procedures are not available for the determination of other nonstarch polysaccharide components (e.g., arabinoxylans, xyloglucans, and arabinogalactans), mainly due to the general complexity of the plant matrix.

4.8.3.2 Enzymatic Chemical Methods Based on GLC

In 1979, a GLC method for the characterization of polysaccharides in soluble and insoluble dietary fiber residues was published [60]. In this method, cellulose was obtained together with lignin (a lignocellulosic residue). The carbohydrate group in Uppsala also independently published a GLC method in the same year in which all individual sugar residues and lignin (i.e., Klason lignin, acid-insoluble residue) were determined. Dietary fiber was calculated as the sum of amylase-resistant polysaccharides and Klason lignin [19]. The latest modification of this GLC method, the Uppsala method [172], for the determination of total dietary fiber as neutral sugar residues, uronic acid residues, and Klason lignin, was adopted as an Official First Action by the AOAC in 1995. It is also approved by the AACC and NMKL in the Nordic countries.

The principles of the Uppsala method for the determination of total dietary fiber are presented in Figure 4.10. Representative milled or homogenized samples are incubated with a thermostable α-amylase (Termamyl®; Novo A/S, Denmark) and amyloglucosidase in aqueous acetate buffer to degrade the starch. This enzyme system, which was introduced in the field of dietary fiber analysis by Theander and Åman in 1979, has proved to be very effective. An important reason for this is that the incubation with Termamyl in a boiling water bath causes simultaneous gelatinization and hydrolysis of the starch, thereby minimizing starch retrogradation. Next, the soluble fiber portion is precipitated in 80% aqueous ethanol, leaving low-
molecular-weight carbohydrates in solution, and the resulting insoluble residue, containing soluble as well as insoluble fibers, is isolated by centrifugation. The content of neutral enzyme-resistant polysaccharides in the residue is determined by conventional sugar analysis: acid hydrolysis and derivatization of monosaccharides released as alditol acetates that are quantified by GLC. The content of uronic acid residues is quantified by colorimetry of the polysaccharide hydrolysate [72], whereas the Klason lignin content is determined gravimetrically as the residue resisting acid hydrolysis and is corrected for its ash content. The analysis of individual sugar constituents gives an indication of the main polysaccharides present in the sample. In wheat bran, for example (Figure 4.11), arabinoxylans are represented by arabinose and xylose, while glucose mainly reflects the content of cellulose. Smaller amounts of mannose- and galactose-containing polysaccharides are also present, whereas rhamnose and fucose are found only in trace amounts.

The enzymes must not contain appreciable fiber-degrading activity during the starch degradation stage, as this will result in underestimated dietary fiber values. This is caused by incomplete precipitation of polysaccharides due to the decrease in molecular weight resulting from enzymatic degradation. All enzyme batches should therefore be checked for the absence of fiber-degrading activity prior to the dietary fiber analysis — for example, on commercially available polysaccharides [172]. It is, however, evident that some enzyme-resistant starch may be present in the residues and included in the dietary fiber analysis [172]. In human diets, which usually have a low content of native lignin, the value for Klason lignin may include, with the exception of lignin

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**FIGURE 4.10** Scheme for analysis of total dietary fiber by the Uppsala method.
Carbohydrates in Food

and modified lignins, unavailable polymers such as cell wall protein (extensin), tannin–protein complexes, and polymers arising from Maillard and caramelization reactions [57]. A clear advantage of the Uppsala method is that the content of individual sugar residues of the dietary fiber polysaccharides is determined. This information can be valuable when the functional and nutritional properties of dietary fiber are investigated or predicted.

Another method based on GLC analysis is that of Englyst [64,77,173] which has undergone a number of developments. This fiber analysis method based on GLC is similar to the Uppsala method in many respects, with the most significant differences emerging from the use of a different definition of dietary fiber (the sum of nonstarch polysaccharides). Samples are treated with DMSO to remove native starch and, when present, resistant starch also. Furthermore, a different enzyme system is used for starch degradation (Termamyl®, pancreatin and pullulanase) and different conditions for the hydrolysis of polysaccharides, with no separate determination of lignin being performed. The limited data available from comparisons of these methods generally indicate good agreement between total dietary fiber determined by enzymatic

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gravimetric methods and the Uppsala method. The Englyst method, however, generally gives lower dietary fiber values in foods containing resistant starch or lignin.

A modification of the Uppsala method using HPLC instead of GLC to measure fiber-derived sugars [174] has been used to determine fiber content and composition for very many different types of food samples. It was found, by comparison with data from the AOAC method, that the AOAC fiber value usually was somewhat higher than that measured with the modified Uppsala method, although data from the two methods were highly correlated [175]. HPLC is a very useful method for the determination of digestible and nondigestible oligosaccharides and small polysaccharides [176].

4.8.3.3 Specific Methods for Determination of Substituents

Common substituents that have been reported for different types of polysaccharides are O-methyl (as ethers or esters), O-acetyl groups, and esterified phenolic acids such as ferulic and p-coumaric acids. Several methods are available for the quantification of the methyl ester content in esterified pectin, and these generally involve alkaline treatment to yield methanol. The methanol can then be quantified colorimetrically [177] or analyzed directly by GLC [178]. The content of O-acetyl groups in a polysaccharide is determined essentially the same way as for the methyl ester content: saponification and quantification of acetic acid released, for example, by colorimetry [179] or by GLC, directly [180] or as 1-acetyl-pyrrolidine [181]. The position of O-acetylation can be determined using the initial protection of free hydroxyl groups by acetyl formation with methyl vinyl ether, followed by replacement of O-acetyl groups by O-methyl groups during methylation and by GLC–MS analysis of sugar residues after hydrolysis and realkylation with, for example, ethyl iodide [182,183]. In order to determine the content of esterified phenolic residues, the polysaccharide material is generally treated with alkali to release the phenolic acids that may then be quantified by GLC [184,185] or HPLC [186,187]. Separation and quantification of p-coumaric and ferulic acids by high-performance thin-layer chromatography has also been reported [188].

4.9 CONCLUSIONS AND FUTURE

In recent years, methods for the analysis of cell wall polysaccharides and associated components in food have rapidly developed. Good methods for the determination of individual neutral sugar components are available, but rapid methods for the determination of individual uronic acid residues are not available today. The basic structures of most major cell-wall polysaccharides are known. In many cases, however, structural heterogeneities make a definite
determination difficult. In plant structures such as the cell wall, non-starch polysaccharides, together with other components, are organized in complex three-dimensional structures that are neither uniform nor completely described. In the future, significant efforts must be dedicated to elucidating the structure and properties of these complexes, as primarily these macromolecular structures, rather than the specific components, determine the technological and nutritional properties of cell-wall polysaccharides in foods. Further development of chemical, spectroscopic, microscopic, and immunological methods will then be a necessity.

REFERENCES


96. Andersson, R., Wheat Flour Polysaccharides and Breadmaking, dissertation, Swedish University of Agricultural Sciences, Department of Food Science, 1993.


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5 Functional Aspects of Cereal Cell-Wall Polysaccharides

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5.1 INTRODUCTION

Polysaccharides serve the growing plant as a structural component that maintains the tissue integrity, as a conduit structure for the movement of water and low-molecular-weight solutes that help maintain osmotic pressure, and as a barrier against microbe and insect penetration [1]. In foods, they control rheological properties, water binding, and the sensory perception of texture, and they are important sources of nutrients and dietary fiber [2]. Because cellulose is a minor component of cereals, this article will focus primarily on pentosans and (1→3),(1→4)-β-D-glucans, referred to here as β-glucans, and their functional aspects in baking, malting, brewing, and animal feeding.

Wheat pentosans consist of soluble and insoluble linear arabinoxylans and branched arabinogalactans. The soluble arabinoxylans are responsible for the high viscosity of their solutions in water [3]. The fundamental rheological properties of wheat and rye soluble arabinoxylans have been the object of numerous investigations during recent years.

The use of endoxylanases with different properties has given new information regarding the important role of soluble arabinoxylans in wheat baking [4]. The high viscosity of pentosan solutions is also considered extremely important for the quality of rye bread. Rye meal contains roughly three times as much water-soluble arabinoxylan as does wheat [5]. In rye dough, the gluten does not form a film, and it has been suggested that the gas retention is due to the ability of soluble arabinoxylans, with their high viscosity, to stabilize gas cells [6]. In beer, pentosans contribute to foam stability, but they may also have undesirable effects on beer filtration and haze formation [7].

Oat bran and oat products have captured the attention of both industry and the scientific community because the soluble β-glucans abundant in oat bran have been shown to have cholesterol-lowering effects in rats and humans [8–10]. Soluble mixed-linkage β-glucan has a high viscosity in water [11,12], and it has been suggested that the creation of viscous conditions within the small intestine is one of the mechanisms involved in the lowering of postprandial blood glucose and insulin levels [13] and in hypocholesterolemic responses to oat and barley in animals and in humans [14–16].

It has been known for long time that barley β-glucan can form a gel [17,18]. Recent studies have shown that low-molecular-weight oat β-glucan will also form a gel [19,20]. Although in Western countries gel formation and high viscosity are considered to be largely beneficial in baking and in human nutrition, in brewing these same properties may decrease the rate of wort
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separation and beer filtration. As well as in wheat, barley, oat, and rye, β-glucans have been reported to occur as cell-wall components in sorghum, millet, and corn [2,21]. β-Glucans may have a much wider occurrence in plant cell walls, but they have remained largely uncharacterized, with the exception of those present in the commercially important cereal grains.

5.2 MICROSTRUCTURE OF BARLEY, OAT, RYE, AND WHEAT GRAIN

The way in which the components of the cell wall interact and are interconnected varies in the kernels of the various cereals. An understanding of the structure is necessary to developing an understanding of the interactions that may be present in the isolated cell-wall components. The aleurone layer of wheat, rye, and oat grain is one cell thick (Figure 5.1, Figure 5.2, and Figure 5.3), and that of barley grain is two or three cells thick (Figure 5.4). The aleurone layer is surrounded by pericarp, under which is found a testa, and then a thin, compressed layer of nucellus. The wheat aleurone layer is from 30 to 70 µm thick [22], and that of oat varies from 50 to 150 µm [23]. Ferulic acid–arabinoxylan complexes are concentrated in the aleurone layer [24,25]. The accumulation of phenolic complexes is important in providing strength.

The staining of the primary cell walls in Figure 5.1 to Figure 5.4 is based on the specific interaction of Calcofluor with mixed-linkage β-glucans, which are the major cell-wall components in barley and oat and minor components in rye and wheat. According to Fulcher and Miller [26], the distribution of β-glucan in oat kernel varies in low and high β-glucan varieties [26]. Although only five oat cultivars were examined, the clear trend was the even

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FIGURE 5.2 Rye kernel. (Legends as in Figure 5.1.)

FIGURE 5.3 Oat kernel. (Legends as in Figure 5.1.)

FIGURE 5.4 Barley kernel. (Legends as in Figure 5.1.)
distribution of β-glucan in the high β-glucan variety and its concentration in the subaleurone layer in the low β-glucan variety. In the case of barley, there is no particular concentration in the subaleurone region, regardless of the β-glucan content. The composition of the aleurone and starchy endosperm cell walls of barley varies considerably. Aleurone cell walls consist of 67% arabinoxylan and 26% mixed-linkage β-glucans, whereas the starchy endosperm cell walls contain about 20% arabinoxylans and 70% mixed-linkage β-glucans [27–31].

In rye, the β-glucan content is much lower [32] and seems to be evenly distributed throughout the grain. In wheat, the highest concentration is in the subaleurone layer with little in the rest of the endosperm [33]. The endosperm cell walls of the mature grain of rice, maize, sorghum, and millet are thinner than those of barley, rye, triticale, and wheat [34].

Primary cell walls (walls of aleurone, starchy endosperm, and scutellum) consist of an amorphous matrix in which cellulose microfibrils are embedded [2]. The cellulosic microfibrils become visible after alkali extraction of the aleurone walls.

The main component of wheat and rye aleurone cell walls is arabinoxylan [31]. In wheat, barley, and rye, ferulic acid is esterified to the arabinoxylans [5,24,25,35,36]. Ferulic acid–arabinoxylan complexes are concentrated in the aleurone layer. Phenolic compounds are autofluorescent, and low magnification reveals the distribution of the phenolics. Treatment of bran with alkali liberates much of the ferulic acid, which reacts with protein to form an undesirable protein–phenol complex [34]. No ferulic acid is associated with β-glucan [36].

Inside the rather thick aleurone cell wall, protein bodies are very densely packed. Each aleurone grain is coated by lipids. Within the protein bodies are regions that are not stained by any protein- or lipid-specific dye [34]. Two separate and chemically distinct structures are embedded in the protein of oat, wheat, barley, and rye aleurone cells [37–40]. These structures are phytin deposits and assemblies of vitamins. Phytin is a salt of myoinositol hexaphosphate and an excellent source of phosphorus. The aleurone layers of barley, wheat, rice, oats, triticale, and rye contain high levels of phosphorus and potassium, whereas those of maize, sorghum, and millet contain significantly lower levels [41]. Dietary phytin can have a negative effect on mineral absorption. Hydrolysis of the complex is required before the minerals become bioavailable. B-vitamin niacin, one of the most important vitamins in the aleurone cells, is associated with a number of chemical components [42–44]. The phenolic-acid-rich cell walls of the aleurone layer are highly stable against food processes and digestion [22,45].

Isolated cell-wall components often contain other chemical components that affect the functional properties. β-Glucans from barley have been shown to contain firmly linked peptide sequences [46]. Soluble arabinoxylan isolated
from wheat or rye grain contains protein and ferulic acid, of which the former has an important role in the interfacial behavior of arabinoxylans and the latter in the oxidative gelation of arabinoxylans [3]. The aleurone layer is an important source of hydrolytic enzymes. β-Endoglucanase, which can persist through the gum extraction procedure, decreases the viscosity of bran water slurries and water solutions of isolated β-glucans [47,48].

5.3 FUNCTIONAL PROPERTIES

5.3.1 PENTOSANS

5.3.1.1 Solubility

The total and water-soluble pentosans comprise about 7 to 8% and 2 to 3%, respectively, of the rye grain [49] and 1.4 to 2.1% and 0.54 to 0.68%, respectively, of the wheat grain on a dry matter basis [50]. The pentosan content of barley grain varies between 4 and 8%, of which up to 75% is present in the husk [51,52]. Barley pentosans are mostly insoluble in water. The pentosan content of oat groats varies between 2.2 and 4.1%, and that of rice between 1.2 and 4.0% [53]. Details of the chemical structure of these components can be found in the previous chapter of this volume.

The pentosans are divided on the basis of solubility into water-soluble (WS) and water-insoluble (WIS) pentosans. The WS pentosans are extractable with cold water, whereas alkali is needed to extract the WIS pentosans. The solubility of the pentosans varies widely with climatic conditions [54]. Because of differences in extraction procedures, comparisons of literature values for solubility and amounts of pentosans extracted may not be very meaningful. The percentage of soluble pentosans is higher in the endosperm than in the bran and shorts fractions [55].

Arabinosyl side chains affect the solubility of the (1→4)-β-xylan, which in the unsubstituted state aggregates into highly insoluble complexes. This has been shown in the preparation of a series of water-soluble arabinoxylans from purified wheat flour arabinoxylan by partial removal of arabinosyl side branches using a β-L-arabinofuranosidase [56]. Rye arabinoxylans can be classified on the basis of extractability: Arabinoxylan I has an arabinose/xylose (Ara/Xyl) ratio of 0.5, and it is totally water extractable; arabinoxylan II has an Ara/Xyl ratio of 1.4, and it is partially water extractable [57,58]. As for arabinosyl side chains, uronic acid, short oligosaccharide, phenolic, and acetyl substituents affect the shape and solubility of arabinoxylan in a similar way [2].

Newer methods of preparing unextractable arabinoxylans have been published [59,60]. The chemical structures of wheat WS and WIS arabinoxylans are basically the same, the WIS having a slightly higher molecular weight [61]. The differences in extractability are probably due to differences in chemical and physical interactions. Substituents such as phenolic acids, acetyl
groups, and proteins may be found in cereal arabinoxylans [2,62–64]. A highly substituted arabinoxylan fraction linked to wheat gluten has recently been found [65,66]. An arabinoxylan-containing extract with exceptional rheological properties has been isolated from wheat bran by extraction with $0.5-M$ NaOH at $25^\circ C$ [67].

### 5.3.1.2 Water-Holding Capacity

It is generally accepted that the pentosans have an excellent water-holding capacity, although few studies have been published on this property. Estimates suggest that the water uptake of pentosans is about 15 g water per g dry pentosans [68]; thus, pentosans have an impact on the water absorption of dough, especially that of rye dough, because the pentosan content of rye is about twice as high as that of wheat [68–72]. Even in a standard wheat baking recipe, the pentosans are able to bind one fourth of the water [68]. In studies of the effect of added WS and WIS pentosans of rye and wheat on the farinograph properties of wheat and rye doughs, the pentosans were found to contribute to the water absorption of both doughs and the dough development time of wheat dough [73,74].

Pentosans form gels with an oxidizing agent. The water-holding capacity of gels made of soluble wheat pentosans depends on the chemical composition and particle size of the dry pentosan powder [3]. The water-holding capacity of oxidized arabinoxylans is clearly greater (75 to 90%) than that of oxidized pentosans that also contain arabinogalactans (40 to 60%). Values of 46 to 68% and 47 to 113% have been reported for arabinoxylan gels with particle size greater than 0.8-mm diameter and 0.4- to 0.5-mm diameter, respectively.

### 5.3.1.3 Surface Activity

Wheat arabinoxylans and arabinogalactans both decrease the surface tension of water [3]. The surface tension vs. concentration for two water-soluble wheat pentosans, arabinoxylans and arabinogalactans, is shown in Figure 5.5. The surface tension of water at $25^\circ C$ is reduced by 20 mN/m, and a constant value is reached at 0.6% (w/v) concentration. Whether this is the property of pentosans or proteins present in the preparations has been discussed by Eliasson and Larsson [75]. They concluded that the curve in Figure 5.5 is typical of uniform and amphiphilic molecules, indicating that not only the arabinogalactans but also the arabinoxylans are truly linked to proteins.

### 5.3.1.4 Molecular Weight

One of the most important parameters characterizing a macromolecule is its molecular weight. At a molecular weight exceeding a critical value, the low shear rate viscosity increases with molecular weight. In practice it has proved
difficult to determine the molecular weight of polysaccharides [76]. Not only are they polydisperse, but they are also nonideal in the thermodynamic sense. It is difficult to define their conformation, and especially at high solute concentrations some polysaccharides self-associate. In many cases, knowledge is required of the molecular weight distribution. Osmometry and classical light scattering provide only molecular weight averages, whereas calibrated gel permeation chromatography provides information about the entire molecular weight distribution. When molecular weight distributions of arabinoxylans are determined using dextran fractions for calibration, these should be regarded as equivalent dextran molecular weights rather than as absolute for the arabinoxylans.

The arabinoxylan from wheat and rye endosperm exhibits a broad molecular weight distribution. For cold-water-soluble rye arabinoxylans, a mean molecular weight of 1 million and for hot-water-soluble arabinoxylans molecular weight values between 620,000 and 970,000 have been reported by using dextran and synthetic amylose standards [5].

Differences in molecular size have been demonstrated for different wheat and rye varieties [3,77]. Weight-averaged molecular weights of rye and wheat pentosans have been reported to be 770,000 and 255,000 to 400,000 [78], respectively, and number-averaged molecular weights have been reported to be 90,000 and 61,000, respectively. Rye pentosans show a higher degree of polydispersity in solution than do wheat pentosans [79].

**FIGURE 5.5** Decrease in the surface tension of water effected by (a) arabinoxylan and (b) arabinogalactan at various concentrations. (From Izydorczyk, M. et al., *Cereal Chem.*, 68, 145, 1991. With permission.)
5.3.1.5 Rheological Properties

5.3.1.5.1 Intrinsic Viscosity

Intrinsic viscosity is a measure of the hydrodynamic volume occupied by a polymer and depends on the molecular weight and conformation of the polymer and on the solvent. Water-soluble wheat arabinoxylan exists in solution as a fully extended rod with an axial ratio of approximately 140 [56]. The arabinosyl substituent has an important effect on the shape of the molecule in aqueous solution. \((1\rightarrow4)-\beta\)-Xylan, which has no arabinosyl residues, forms a threefold, left-handed helix and exists as a fully extended “twisted ribbon” in the solid state [80,81]. With removal of arabinosyl substituents, the polymers appear to become more flexible. After more extensive removal, the unsubstituted xylan chains form stable, interchain associations.

The intrinsic viscosities of water-soluble nonstarch polysaccharides (NSPs) and soluble arabinoxylans (SAXs) and arabinogalactans (SAGs) vary for rye, wheat, and triticale, as shown in Table 5.1. The intrinsic viscosity of arabinoxylans varies from one wheat flour to another. Values from 1.9 to 6.9 dl/g have been reported for wheat arabinoxylans and 0.045 to 0.062 dl/g for wheat arabinogalactans, indicating that arabinoxylan is the main contributor to the high viscosity of pentosans in aqueous solution [3,56].

When soluble arabinoxylans from different wheat varieties were fractionated by the graded \((\text{NH}_4)_2\text{SO}_4\) technique into three polymeric fractions differing in molecular size and fine structure, most of the arabinoxylans were found in the high-molecular-weight fraction; however, the yields of the three fractions differed among the varieties. The intrinsic viscosity of the highest molecular

<table>
<thead>
<tr>
<th>Sample</th>
<th>Intrinsic Viscosity (η)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat NSP</td>
<td>1.7</td>
<td>82</td>
</tr>
<tr>
<td>Wheat SAX</td>
<td>6.1</td>
<td>56</td>
</tr>
<tr>
<td>Wheat SAX</td>
<td>2.8–4.2</td>
<td>3</td>
</tr>
<tr>
<td>Wheat SAX</td>
<td>3.2–6.9</td>
<td>78</td>
</tr>
<tr>
<td>Wheat SAG</td>
<td>0.05–0.06</td>
<td>3</td>
</tr>
<tr>
<td>Rye NSP</td>
<td>5.9</td>
<td>82</td>
</tr>
<tr>
<td>Triticale NSP</td>
<td>4.0</td>
<td>82</td>
</tr>
</tbody>
</table>

*a NSP, nonstarch polysaccharides; SAX, soluble arabinoxylans; SAG, soluble arabinogalactans.
weight fraction varied between 3.15 and 5.24, that of the medium molecular weight fraction between 2.23 and 3.60, and that of the lowest molecular weight fraction between 0.88 and 1.21 for the different varieties. The high intrinsic viscosity of soluble arabinoxylan correlated with a high xylose/arabinose ratio and high ferulic acid content [83]. A recent study showed that water-soluble arabinoxylans from wheat flour can be fractionated by graded ethanol precipitation [78]. The ferulic acid content was lower in fractions precipitated at a high ethanol percentage. Both ferulic acid monomers and dimers were present. The content of ferulic dimers might explain variations in the macromolecular properties.

Rye arabinoxylans have been studied to a much lesser extent than wheat arabinoxylans. Whereas the intrinsic viscosity of the wheat arabinoxylan is independent of NaCl concentration, the rye NSP showed significantly higher viscosity in 0.1-M NaCl than in 0.01-M NaCl solution [82]. Compared to the intrinsic viscosity of other polysaccharides, the arabinoxylans show a value similar to that of guar gum (2.3 to 6.8 dl/g) but are more viscous than dextran (0.214 dl/g) and gum arabic (0.12 to 0.25 dl/g) [84].

5.3.1.5.2 Flow Behavior

At a low polymer concentration, zero-shear-rate viscosity (η₀) increases with increasing concentration, and η₀ vs. concentration has a slope of 1.3 [85]. With a further increase in concentration, the slope changes to a value of about 3.3, reflecting the onset of coil overlap between the polymer chains. The critical concentration at which the transition occurs depends on the volume occupied by each molecule.

Two critical concentrations were observed for wheat arabinoxylans as shown in Figure 5.6 [86]. In contrast to flexible random coil polysaccharides, an intermediate zone was found between the dilute and concentrated domains. The first critical concentration (c*) is related to the onset of coil overlap. Beyond the second critical concentration (c**) the chain dimensions become independent of concentration. The result indicates that wheat arabinoxylans also assume a fully extended rod-like shape in solution, as suggested earlier by Andrewartha and coworkers [56]. A more recent study, however, showed that wheat arabinoxylans behave as random coils and are semiflexible. The rigidity did not change with substitution degree in the range of 0.39 to 0.93 [78].

At low shear rates, wheat and rye arabinoxylans exhibit Newtonian behavior (Figure 5.7). The zero-shear-rate viscosity of rye and wheat arabinoxylans is almost the same when compared at the same arabinoxylan concentration. In the case of wheat arabinoxylans, the shear rate at which the apparent viscosity begins to decrease depends on the molecular weight. In the order of decreasing molecular weight, the critical shear rate shifts toward higher shear rates [86]. Viscosity measurement showed that the arabinoxylan I fraction isolated from cultivar Muskate had a higher viscosity than the corresponding
fraction from cultivar Danko [87]. This difference was due to a much higher proportion of high-molecular-weight polymers in Muskate than in Danko.

Flow curves have been described by using a power law equation:

\[ \sigma = K \gamma^n \]

where \( \sigma \) is the shear stress, \( K \) is the consistency index, \( \gamma \) is the shear rate, and \( n \) is a dimensionless constant that indicates deviation from Newtonian flow (\( n = 1 \) for Newtonian flow). The \( n \) values (0.16) are lower for high-molecular-weight arabinoxylans. Shear thinning behavior has been observed for a polymer concentration as low as 0.2% (w/v). Compared with 1.5% wheat arabinoxylan, the viscosity of 1.5% rye arabinoxylan decreases slightly more slowly with increasing shear rate (Figure 5.7). One clear difference between the samples is the xylose/arabinose ratio, which is 1.45 for wheat [86] and 2.03 for rye arabinoxylan.

5.3.1.5.3 Viscoelastic Behavior

In the case of a dilute polysaccharide solution, the elastic stress relaxes and the viscous stress dominates (loss modulus \( G'' \) > storage modulus \( G' \)).
particularly at low frequencies. In the case of a gel system, in which the junction zones are stable on a relatively long time scale, the stress cannot relax, and both moduli are more or less independent of frequency; $G'$ is greater than $G''$ throughout the commonly used frequency range ($10^{-2}$ to $10^{2}$ rad$^{-1}$). Storage modulus is a good indicator of gel rigidity. Tan$\delta$, which describes the relative changes in $G'$ and $G''$, is very large (>1) for a fluid system. Dynamic measurements are particularly useful in monitoring the gel formation of polymers. Dynamic viscoelastic measurements can be made at such small deformations that the effects on structure are negligible. When a gel is formed, $G'$ increases (as does $G''$, but to a more limited extent), and consequently tan$\delta$ decreases. Recent reviews have discussed the application of small-deformation rheological testing to the characterization of biopolymer networks [85,88–90].

Recently, a study was made of the frequency dependence of the storage ($G'$) and loss ($G''$) moduli of various wheat arabinoxylans of different molecular size [86]. Only fractions with higher molecular weights were found to exhibit elastic properties. The frequency at which $G'$ and $G''$ crossed shifted to higher frequencies with lower concentrations and molecular weights.

5.3.1.5.4 Oxidative Gelation

Arabinoxylans containing ferulic acid form gels upon the addition of oxidizing agents. Hydrogen peroxide is effective, working together with the peroxidase that flour contains naturally. No heat treatment is required. Several hypotheses
have been suggested for the oxidative gelation mechanism: (1) Arabinoxylan ferulic acid is linked to tyrosine residues or to ferulic acid complexed with protein [91]; (2) arabinoxylan ferulic acid is linked to N-terminal protein groups; (3) part of ferulic acid is bound to the glycoprotein and serves as a bridge between the protein and pentosan [72]; or (4) a covalent binding of protein with the arabinoxylan chain via a ferulic acid group is involved [64].

All the data make it clear that ferulic acid plays an important role. Only a few oxidizing agents have the ability to induce gelation [64]. These include ammonium persulfate, formamidine disulfide, and hydrogen peroxide in the presence of peroxidase, which causes the formation of free radicals. Potassium bromate and ascorbic acid, two oxidizing agents commonly used in bread, do not cause gelation.

A similar oxidative gelation process has been reported for soluble arabinoxylans of rye [92]. It has not been confirmed that protein has a role in the gelation, but experiments involving inhibitors of the oxidative gelation process suggest that the aromatic ring and not the propenoic moiety is involved in the reaction of ferulic acid. While the findings are in agreement with those of Moore and coworkers [93] for wheat arabinoxylans, they are contrary to the results reported by Hoseney and Faubion [64].

The gelling ability of arabinoxylan has been studied by small-amplitude shear strain oscillatory testing [86,94]. When WS wheat arabinoxylans with high molecular weights are treated with horseradish peroxidase and H2O2, a rapid rise in storage modulus ($G'$) occurs [94], followed by a plateau. A correlation exists between the value of the plateau storage modulus and the intrinsic viscosity, indicating that the ability of arabinoxylans to form gels is dependent on their molecular weight [86]. $G'$ values of high-molecular-weight fractions increase rapidly with polymer concentration, whereas those of low-molecular-weight fractions show a much weaker concentration dependence. A typical frequency sweep of the moduli ($G'$, $G''$) and dynamic viscosity ($\eta$) for a high-molecular-weight fraction is presented in Figure 5.8. $G'$ is seen to be greater than $G''$ at all frequencies [94].

For smaller molecular fractions, $G''$ exceeds $G'$ at all frequencies, and the moduli are highly dependent on frequency, indicating a liquid-like behavior. Storage modulus values reported for 1% arabinoxylan gels are in the range of 0.05 to 61.40 Pa (measured at 1.0 Hz and 4% strain), which means that soluble wheat arabinoxylans form rather soft gels [86,94].

The most important gel properties are water-binding capacity and gel strength. The term “gel strength” often is used to describe both the water-binding and texture, even though the two properties are not always correlated [95]. Although the gels are very soft, they nevertheless have a large capacity to bind water.

The fractions Izydorczyk and Biliaderis [86] studied differed in molecular weight, chemical structure, and ferulic and protein contents, and the contribution
of the separate characteristics to the rheological properties of the oxidized gels remains unclear, as does the question of whether or not the presence of protein is necessary for the gelation to occur.

Water-insoluble arabinoxylans reportedly form a gel under the same conditions as water-soluble arabinoxylans [96]. It is likely that the isolation procedure and the heating of the WIS pentosan dispersion in boiling water modify the WIS pentosans; that is, pentosans insoluble in their native form become soluble. Otherwise, it is difficult to understand how such oxidation can occur.

5.3.1.6 Significance in Baking

Pentosans influence baking in at least two ways: (1) Because of their significant water-holding capacity, they affect the water distribution in the dough. This effect is important in both wheat and rye baking processes. (2) Through their rheological properties they also affect the gas retention of dough, particularly rye dough. Whereas the ability of wheat dough to retain gas is primarily

FIGURE 5.8 Storage ($G'$) and loss ($G''$) moduli and dynamic viscosity ($\eta$) vs. frequency for wheat arabinoxylan after 1 hour of treatment with peroxidase (0.22 PU/ml) and H$_2$O$_2$ (1.5 ppm) at 15°C. (From Izydorczyk, M.S. and Biliaderis, C.G., Carbohydr. Polym., 17, 237, 1992. With permission.)
associated with gluten, in rye dough gas retention is attributed to the high viscosity of soluble arabinoxylans [6]. Conclusions regarding the role of oxidative gelation in the mechanism by which pentosans improve the loaf volume are contradictory [97,98]. Because hydrogen peroxide is required for oxidative gelation but is not used in rye baking, oxidative gelation cannot be involved in the rye baking process.

The impact of WS pentosans on the bread-making potential of wheat and rye flour has been studied in reconstitution experiments [73,98–101], by depolymerizing pentosans in situ with highly purified xylanase [4,102–104], by selecting a flour set with wide variations in many parameters and utilizing multivariate tools for correlation analysis [105], and by adding pentosans to a flour as a function of water content and mixing time [70,106].

Although it is generally accepted that pentosans influence the water absorption of doughs [68–72], the results describing their impact on the loaf volume of wheat and rye breads are contradictory. Some reasons for the discrepancies in results include: (1) The pentosan fractions have not been characterized [75], (2) the effect of added pentosans has not always been evaluated as a function of water content and mixing time [106], and (3) most of the experimental strategies used to study the influence of pentosans on baking properties induce other changes in the dough.

5.3.1.6.1 Rye Baking

It is generally accepted that pentosans play an important role in rye baking by influencing the viscosity and gas-retaining ability of the dough [72]. Viscosity determines dough yield, stability, and volume of the dough and bread loaf [54]. Highly viscous doughs will yield more dough by retaining more water; they will have better stability, but the loaf volumes will be lower.

In the rye baking process employed by most of the bread industry in Scandinavia, a period of about 5 hours elapses from the start of a dough mix until a loaf of bread is baked. The enzymes present in rye flours and the additional effect of various degrading enzymes produced by acid-forming microorganisms can cause a decrease in the viscosity, with the result that the dough is too fluid to process into bread. The degradation of cell walls can be controlled by flour particle size, dough temperature, pH, and salt concentration.

Optimal results in rye baking are achieved at a definite pentosan/starch ratio of 1:16 [107,108]. Because starch is the major component of rye flour, starch-degrading enzymes play a key role in the baking quality. In sprouted grain, α-amylase activity is very high in the aleurone layer [54], and, if the aleurone cell walls degrade during the baking process, the same amylolytic activity will have that much greater an effect on starch hydrolysis. In Scandinavian-type rye bread, which is made with the whole meal, the degree of degradation of aleurone cell walls has been observed to differ with the rye variety [32].
Particle size distributions produced in milling may vary with the variety. Rye doughs and breads made from whole meals contain large and rigid particles, which decrease the extensibility of the dough and affect the gas cell structure [32]. The particles originating from bran have a very great impact on the hardness of rye bread. The hardness of rye bread baked from whole-meal flour can often be in the range of 900 to 2000 g, whereas that of rye bread baked from endosperm flour is about 400 g [109,110]. Water-soluble pentosans have been shown to improve the texture of rye baked products, whereas water-insoluble pentosans have a negative impact [72]. Good baking quality of mill streams can only be achieved if the pentosan/starch ratio is correct.

5.3.1.6.2 Wheat Baking

The role of pentosans in wheat baking is regarded as minor. Reconstitution experiments have shown that the WS pentosan fractions of both rye and wheat have a volume-enhancing effect on gluten-starch loaves [72,98,101]. The protein moiety present in most pentosan fractions studied is not regarded as important because enzymatic degradation of the protein has no effect on loaf volume, nor does the removal of ferulic acid have any effect. This suggests that oxidative gelation is not involved in the improvement of loaf volume by pentosans; rather, the action of WS pentosans would appear to be related to an increase in dough viscosity, because the addition of oat β-glucan, corn bran hemicellulose, and xanthan gum to gluten-starch doughs also increases the loaf volume [98].

In experiments carried out by Vanhamel and coworkers in which rye WS pentosans were added to wheat flour, the mixing time and amount of water added were found to influence the loaf volume; also of relevance were the mode of addition, whether in solution or as dry powder [106]. The addition of 2% high-molecular-weight pentosans generally increased the loaf volume and improved the crumb structure. The maximum loaf volume was obtained at several combinations of absorption levels and mixing times. When the rye WS pentosans were presoaked before addition, maximum loaf volume was obtained only at high absorption levels and low mixing times.

In an investigation involving a sample set of 100 wheat samples with documented baking and dough properties, Andersson [105] showed that water absorption as determined by a farinograph could be explained by the content and composition of arabinoxylans in the flour; however, bread loaf volume was not significantly predicted. By using two endoxylanases with selectivity either for water-unextractable or water-extractable pentosans, Courtin and coworkers [4] showed that water-soluble pentosans with medium and high molecular weights have a positive impact on loaf volume, whereas insoluble pentosans have detrimental effects. In whole-meal wheat breads, high proportions of insoluble tissue particles interfered with the protein network [111].
5.3.1.6.3 Effect on Bread Staling

Rye bread stales at a much lower rate than wheat bread, and it has been suggested that the WS and WIS pentosans retard the increase in crumb firmness [71,112]. X-ray measurements of bread have shown that crystallization of starch is involved in the staling of bread [113]. The effect of soluble arabinoxylans on the retrogradation of starch gels has been studied by differential scanning calorimetry (DSC) [114,115] and dynamic viscoelastic measurements [115].

Gudmundsson and coworkers [115] have shown that the positive effect of pentosans during the aging of starch gels is based on their ability to absorb and control the effective water content available to starch. The rate of amylopectin crystallization in starch gels containing soluble arabinoxylans is either increased or decreased depending on the final water content of starch, because the recrystallization of amylopectin is highly dependent on the water content [116,117]. Retrogradation is minimal in starch gels with moisture content less than 20% but increases significantly between 20 and 30%.

The moisture content also regulates the firming of bread. Because the firming rate is highest in bread with a moisture content of 20%, in which starch retrogradation does not play an important role, the firming must be due to other mechanisms, as well. Rogers and coworkers [118] found the firming rate to be lower in breads with 35 and 37% moisture content than in control bread (31% moisture). The higher moisture content of rye bread than of wheat bread may be one explanation for the lower staling rate of rye bread.

5.3.1.7 Significance in the Malting and Brewing Process

Arabinoxylans have received little attention in studies on the effects of biopolymers in malting and brewing, even though they are the major polysaccharides in the barley aleurone cell walls [31]. Most of the arabinoxylans present in barley are water insoluble [119]. Like the β-glucans, which have been studied extensively, arabinoxylans tend to decrease the filterability of beer [120–122] and cause the formation of haze [123]. High levels of pentosans have been found in hazes of beers made from grists, and high-molecular-weight pentosans have been shown to correlate significantly with beer filterability [124].

Whereas β-glucans are degraded extensively during malting, pentosans are not [34,125]. Only a few of the enzymes from the malt itself show activity toward arabinoxylans [126]. Parkkonen and coworkers [32] have shown that incubation of rye kernel sections with endo-β-glucanase results in the disappearance of Calcofluor-stained β-glucan cell walls, whereas endoxylanase is able to degrade only a small portion of the kernel cell walls. The use of endoxylanase leaves large amounts of high-molecular-weight arabinoxylans if the arabinoxylan has a high degree of substitution [119].
When wheat, barley, or sorghum is used as an adjunct, pentosan-degrading enzymes must be added as well. The addition of endo-β-xylanase has been shown to decrease the wort viscosity, and the final viscosity is independent of the β-glucan concentration [119,127]. Total degradation of arabinoxylans to small oligomers would require the use of an arabinofuranosidase to remove arabinose from the xylan backbone. Bamforth [7] suggests that certain amounts of nonstarch polysaccharides improve the sensoric properties of beer and the stability of beer foam. Extensive degradation of pentosan may have a negative effect on the beer quality.

5.3.1.8 Significance in Animal Feeding

Water-soluble rye arabinoxylans increase digesta viscosity and in that way decrease the absorption of all nutrients in the chick [128–133]. The use of cell-wall-degrading enzymes as a means of improving the nutritive value of rye has been more successful with chickens than pigs [133–137]. Pentosanases in rye-based diets were not effective in increasing pig performance [138]. Because of the thick aleurone cell walls, the bran fraction is of poor nutritional value relative to shorts and middlings [139]. The in vitro digestibility increases significantly if the aleurone is finely ground [140]. The digestion of wheat aleurone cells is much less efficient in chickens than in pigs, however [141,142].

5.3.2 β-Glucans

The β-glucan contents of groats of European and American oat varieties are reported to range from 3.2 to 6.2% [143] and 3.9 to 6.8% [144], respectively. The β-glucan contents of hulled malting and feed barley grains typically range from 3 to 7% [145]; however, as much as 14 to 16% β-glucan has been reported for some cultivars [146]. Whole rye grain contains 1 to 2% mixed-linkage β-glucans, and wheat contains 0.5 to 1.0% [32,53]. It may be that minor amounts of arabinosyl or xylosyl residues are covalently linked with (1→3),(1→4)-β-glucans [147]. Details of the chemical structure of these components can be found in the previous chapter of this volume.

5.3.2.1 Solubility

The solubility of β-glucan is dependent on the fine structure. Most water-soluble β-glucans contain approximately 30% (1→3) and 70% (1→4) linkages, which are organized into blocks of two or three (1→4)-linked residues separated by single (1→3)-linked residues [148]. The higher solubility of mixed-linkage β-glucans than cellulose is due to the presence of (1→3)-β-bonds, which introduce irregularity into the structure. Lichenin, (1→3),(1→4)-β-glucan from Iceland moss (Cetraria islandica), has few blocks of adjacent (1→4) linkages in the polysaccharide chain relative to water-soluble barley...
β-glucan, and the result is a regular shape, which permits more extensive aggregation and gives it poor solubility in water [149]. \((1\rightarrow3)\)β-D-glucan forms a helix [150], which at a degree of polymerization comparable to barley β-glucan is insoluble in water [151].

Evidence for the occurrence of two or more adjacent \((1\rightarrow4)\) linkages has been reported for barley [151,152] and oat \((1\rightarrow3),(1\rightarrow4)\)-β-D-glucans [153]. The \((1\rightarrow4)\) linkages render the polysaccharide more insoluble, and the differences in the number of consecutive \((1\rightarrow4)\) linkages may explain the differences in solubility of some β-glucan preparations [154–156]. Results describing the occurrence of consecutive \((1\rightarrow3)\)-linked units in barley and oat β-glucan are contradictory [151]. Possibly, insufficient enzymatic action during malting and mashing results in incomplete breakdown of the mixed-linkage β-glucans and produces molecules of similar linkage patterns, which have a high tendency to associate and precipitate [34].

While bound to protein, the mixed-linkage β-glucans are insoluble. An enzyme, acidic carboxypeptidase, which is present in raw barley, solubilizes the water-insoluble β-glucan in barley [157]. Comparison of the solubility of β-glucans in different cereals is hampered by variations in the experimental conditions and particle size. Table 5.2 presents some relevant results. In general, the order of solubility of β-glucans is oat > barley > wheat [158]. Solubility seems to correspond with the ratio of cellotriosyl to cellotetraosyl units in the cereal β-glucan structure (approximately 4, 3, and 2 for wheat, barley, and oat β-glucan, respectively) and with molecular weight [67,159]. Drying of isolated barley cell walls and isolated β-glucan preparates reduces the solubility [160]. The oat β-glucan in the thinner inner endosperm cell walls is more soluble than that in the thick subaleurone cell walls [48,161]. Alkali at 50 to 60°C is commonly used [162,163]. The differences in solubility observed during extraction may be due to both molecular weight [154] and cell-wall organization [158].

### TABLE 5.2

<table>
<thead>
<tr>
<th>Sample</th>
<th>Temperature (°C)</th>
<th>Number of Extractions</th>
<th>Liquids:Solids</th>
<th>Percent Soluble (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oat</td>
<td>65</td>
<td>4 × 30 min</td>
<td>30:1</td>
<td>27</td>
</tr>
<tr>
<td>Barley</td>
<td>65</td>
<td>4 × 30 min</td>
<td>30:1</td>
<td>24–65</td>
</tr>
<tr>
<td>Oat</td>
<td>100</td>
<td>3 × 10 min</td>
<td>60:1</td>
<td>63</td>
</tr>
<tr>
<td>Oat</td>
<td>38</td>
<td>1 × 120 min</td>
<td>75:1</td>
<td>63</td>
</tr>
<tr>
<td>Barley</td>
<td>38</td>
<td>1 × 120 min</td>
<td>75:1</td>
<td>78</td>
</tr>
<tr>
<td>Oat</td>
<td>100</td>
<td>1 × 60 min</td>
<td>50:1</td>
<td>43</td>
</tr>
</tbody>
</table>

Water-soluble β-glucans extracted at 40°C account for up to 20% of the total β-glucan in barley endosperm cell walls [27], but 50 to 70% when extracted at 65°C [29,164,165]. By contrast, the β-glucans of wheat are unextractable in water at 65°C [155]. Many breweries use a temperature of 65°C for the extraction of malt in the mashing phase. The percentage of water-soluble β-glucan extracted into 65°C acid buffer is often reported for barley, as this is a useful indicator of malting quality. In poor malting barley, up to 65% of the total β-glucan is soluble in 65°C acid buffer, compared with only about 25% in good malting barley [166].

Woodward and coworkers found (1→3),(1→4)-β-glucan extracted from barley flour at 40°C to have relatively more blocks of three or more adjacent (1→4) linkages than that extracted at 60°C [156]. The authors suggested that small differences in the fine structure determine the solubility; however, the fractions also differed in protein and uronic acid levels, so the contribution of different factors to the solubility remains unclear.

5.3.2.2 Water-Binding Properties of Oat Bran

The most important functional property of oat bran is its high water-binding capacity. Whole oat meal, oat bran, and oat flour have a higher water-binding capacity than wheat, corn, or rice flours. Table 5.3 shows the water hydration capacity of some cereal fibers. Each bran was ground, and the entire sample was sieved through an 80-mesh screen.

5.3.2.3 Molecular Weight

The molecular weight is a fundamental parameter characterizing a polysaccharide and determining its rheological properties. Molecular weight distributions
Functional Aspects of Cereal Cell-Wall Polysaccharides

Table 5.4 illustrates the variation in molecular weights obtained by this method for mixed-linkage β-glucans. This large variation is due to the diversity of the methodology used for the determination of molecular weight and the extraction protocol (solvents, conditions, and sample history). In the case of oat and barley flours, β-glucan extraction procedures that do not inactivate the enzymes will cause a decrease in the molecular weight [167]. In addition, low-molecular-weight β-glucans in solution exhibit fairly rapid aggregation, which can occur during extraction [159].

<table>
<thead>
<tr>
<th>Method</th>
<th>Source</th>
<th>Molecular Weight</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sedimentation velocity osmometry</td>
<td>Barley</td>
<td>200,000</td>
<td>151</td>
</tr>
<tr>
<td></td>
<td>Oat</td>
<td>71,900</td>
<td>168</td>
</tr>
<tr>
<td></td>
<td>Oat, Donald</td>
<td>3 million</td>
<td>169</td>
</tr>
<tr>
<td>Gel permeation chromatography</td>
<td>Oat</td>
<td>49,000</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Oat, Marion</td>
<td>2.9 million</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Oat, Tibor</td>
<td>1.2 million</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Oat, Betha</td>
<td>2.2 million</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Purified oat β-glucan</td>
<td>363,000</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Purified oat β-glucan&lt;sup&gt;a&lt;/sup&gt;</td>
<td>180,000 – 850,000</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>Purified oat β-glucan&lt;sup&gt;b&lt;/sup&gt;</td>
<td>35,000 – 250,000</td>
<td>159</td>
</tr>
<tr>
<td></td>
<td>Oat, Donald</td>
<td>2.9 million</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Oat, Marion</td>
<td>3.0 million</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Oat, Tibor</td>
<td>3.0 million</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Barley, Bruce</td>
<td>2.7 million</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Barley, Rodeo</td>
<td>1.9 million</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Barley, Birka</td>
<td>2.5 million</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Barley, Mingo</td>
<td>1.7 million</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Barley malts</td>
<td>1–1.5 million</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Barley β-glucan</td>
<td>195,000</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Barley β-glucan&lt;sup&gt;b&lt;/sup&gt;</td>
<td>40,000–250,000</td>
<td>170</td>
</tr>
<tr>
<td></td>
<td>Rye</td>
<td>1.1 million</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Oat&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.5 million</td>
<td>171</td>
</tr>
<tr>
<td></td>
<td>Oat&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.1 million</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Oat&lt;sup&gt;e&lt;/sup&gt;</td>
<td>370,000</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Water extraction at 47°C.

<sup>b</sup> Acid hydrolysis.

<sup>c</sup> Cold-water wet-milled.

<sup>d</sup> Ethanol–water wet-milled.

<sup>e</sup> Hydrolyzed, cold-water wet-milled.
Size-exclusion chromatography with high-performance gel column and Calcofluor postcolumn detection has been used to estimate the molecular weight of mixed-linkage $\beta$-glucans in oat bran and groat extract, in pilot and bench oat gum, and in different oat and barley varieties, malt, and rye [168]. As shown in Table 5.4, the molecular weight reported for pilot oat gum is clearly lower than that reported for bench oat gum, probably due to the high shear rates achieved by the disk centrifuge used during purification. A marked decrease in the molecular weight also occurs during purification of oat or barley $\beta$-glucan, but no explanation has been found for this decrease. $\beta$-Glucan isolated from cold-water, wet-milled, fiber-rich oat bran is of higher molecular weight than that isolated from ethanol–water, wet-milled oat bran [171]. The $\beta$-glucans of oat have the highest molecular weights, followed by those of barley, malt, and rye. Although commercial pullulan standards have been reported to be well suited for calibration of $\beta$-glucans [168], they in fact lead to overestimation of the molecular weight. The alternative is to use $\beta$-glucans with different molecular weights as standards [169,171].

In total intensity light-scattering studies of low polydispersity $\beta$-glucan fractions, Vårum and coworkers [172] showed that about 10% of oat $\beta$-glucan forms labile aggregates that are not separated from the nonaggregating component of the $\beta$-glucan by size-exclusion chromatography but which gradually disappear by dilution. Vårum et al. suggest two possible mechanisms: (1) The small fraction of longer (1→4)-$\beta$ linkages in the polysaccharides offers sites for the essentially insoluble cellulose-like sequences of the monomers to associate through crystalline aggregation, or (2) some fraction of the $\beta$-glucan chains may be associated with lipid in the form of lipopolysaccharide (LPS) in a micellar fashion.

5.3.2.4 Rheological Properties

5.3.2.4.1 Intrinsic Viscosity

The intrinsic viscosity of $\beta$-glucan depends on the extraction conditions. At pH 9.2 and extraction temperatures of 90 and 70°C, values for oat $\beta$-glucans are about 13 and 12.5 dl/g, respectively [173]. The intrinsic viscosity values of oat $\beta$-glucans will increase with increased molecular weight. For oat $\beta$-glucans extracted at 47°C by water, values of 0.67 to 3.83 dl/g were reported for the oat $\beta$-glucan samples with molecular weights ranging from 35,000 to 250,000, and values of 4.9 to 6.4 dl/g were reported for oat $\beta$-glucans with molecular weights ranging from 250,000 to 780,000 [20]. The corresponding values for barley (1→3),(1→4)-$\beta$-glucan extracted at 40°C and 65°C are 6.90 and 4.04 dl/g, respectively [134,143], and, for acid-hydrolyzed samples, 0.63 to 3.01 dl/g [20]. The great differences in the intrinsic viscosities of barley and oat $\beta$-glucans is due to different extraction conditions and sample history.

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The chemical structure of the β-glucan is of great importance to the conformation the molecule adopts in solution. Schematic drawings of β-glucans with (1→3); (1→4); and (1→3),(1→4) substitution [174,175] are shown in Figure 5.9. (1→3),(1→4)-β-Glucan exists in extended and flexible conformation in aqueous solution [2].

The specific viscosity of oat β-glucan as a function of concentration gives a curve with three different concentration domains [20]. The first transition is related to initial contact between the individual coils, the second to the intermediate line between the diluted and concentrated domains to rod-like conformation, and the third to interpenetration of the polymer coils. Differences in the critical concentrations among the samples could be explained in terms of molecular weight.

### 5.3.2.4.2 Flow Behavior

The flow properties of oat β-glucans have been studied with the help of rotational viscometers and the power law equation [11,12,176]. Table 5.5 shows the power law constants of a β-glucan solution at 25°C as a function of concentration. An $n$ value of 0.66 and a $K$ value of 3.2 have been reported for a 0.8% β-glucan sample prepared in a pilot plant, indicating degradation due to the much higher shear rates [177] than are available in a viscometer. It is possible that the high shear rates were achieved in a disk centrifuge during purification. Oat β-glucans exhibit strong shear thinning behavior in the shear rate range of 20 to 1600/s but no thixotropy. The shear thinning is greater at higher concentrations [19].

The viscosity of β-glucans decreases strongly during heating but is recovered during cooling. Oat β-glucans are more stable than guar gum or carboxymethylcellulose (CMC) against heat treatment [12]. Solutions of barley β-glucans are stable at 100°C for up to 108 hours [178]. The viscosity of β-glucan solutions may be decreased if the preparation is heated (85 to 115°C) prior to dissolution [179].
In two studies on the effect of other solutes on β-glucan flow behavior, no effect was found for NaCl (0 to 10%) (12,178). At 0.5% β-glucan concentrations, sucrose increases the low-shear-rate (2 to 14/s) viscosity by up to 50% [12], and at 1.5% concentration it decreases the high-shear-rate (600 to 2400/s) viscosity. At high β-glucan concentrations, sucrose may restrict hydration and extension, thus reducing the viscosity. In dilute solutions, increased interchain interactions may compensate for a reduction in hydration.

The usefulness of the power law has been criticized [180]. Certainly a better description of flow properties would require measurements over a wide range of shear rates. Zero-shear-rate viscosity ($\eta_0$) is an important parameter because it is proportional to molecular weight; $\eta_0$ can be determined by various techniques, as shown in Figure 5.10 [181].

Oat β-glucan preparations extracted by water at 47°C showed very different flow properties in comparison to those extracted by alkali [20]. For a sample with molecular weight of 780,000, shear-thinning behavior was observed at concentrations of 4 to 10%, whereas at 1 to 2% concentrations the flow behavior was Newtonian. With lower molecular weights, a decrease in viscosity and shear-thinning properties has been observed. Vaikousi and coworkers [170] studied the flow properties of low-molecular-weight (40,000 to 250,000), acid-hydrolyzed barley β-glucan preparations. At a 1% concentration, all samples exhibited Newtonian behavior. With increased concentration, the solutions showed shear-thinning behavior. Even at 10%, a limiting Newtonian viscosity was observed in the low-shear-rate region.

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---

**TABLE 5.5**

Power Law Constants of β-Glucan Solution at 25°C

<table>
<thead>
<tr>
<th>β-Glucan Concentration (%)</th>
<th>$n$</th>
<th>$K$ (Ns/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.16</td>
<td>0.84</td>
<td>0.04</td>
</tr>
<tr>
<td>0.36</td>
<td>0.62</td>
<td>0.57</td>
</tr>
<tr>
<td>0.54</td>
<td>0.50</td>
<td>3.40</td>
</tr>
<tr>
<td>0.71</td>
<td>0.30</td>
<td>12.2</td>
</tr>
<tr>
<td>0.80</td>
<td>0.28</td>
<td>24.2</td>
</tr>
<tr>
<td>0.88</td>
<td>0.24</td>
<td>36.1</td>
</tr>
<tr>
<td>0.98</td>
<td>0.20</td>
<td>46.9</td>
</tr>
</tbody>
</table>

5.3.2.4.3 Viscoelastic Behavior

The mechanical spectra of β-glucan solutions are typical of concentrated solutions: At low frequencies, $G'' > G'$, and at high frequencies $G' > G''$; both moduli increase with frequency [181]. As the polymer concentration is increased, the transition from solid- to liquid-like responses moves to lower frequencies, a characteristic property of solutions in which the rheology is mainly governed by the degree of entanglement of macromolecules. This is in agreement with the results of Doublier and Wood [19].

Low-shear-rate viscosity and viscoelastic measurements have shown that oat β-glucan is rheologically very similar to guar gum and behaves like a random-coil, nongelling polymer in aqueous solution at concentrations between 0.1 and 2%. Many commercially available food hydrocolloids were studied for synergism with β-glucans, and synergism was found only for carboxymethylcellulose [178].


**FIGURE 5.10** Shear, dynamic, and complex viscosities of β-glucan water solutions.

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5.3.2.4.4 Gels
Low-molecular-weight barley and oat β-glucans in solution aggregate rapidly, leading to a network structure [19,20,159,182]. The gel formation potential and conditions promoting the gel formation have been studied for barley β-glucan because gelatinous precipitates decrease the filterability of beer [18,183,184]. Such precipitates appear when malt is unmodified or when barley adjuncts are added [184]. β-Glucans extracted at mash temperatures above 65°C have significantly reduced gel-forming properties compared with β-glucan extracted below 65°C [183]. The nature of the β-glucan is always important, as the undegraded polysaccharide of barley does not form a gel [7]. Wort and beer β-glucans show similar gel potential after they have been isolated and dissolved in water, although gel formation is less in wort than in beer [17]. This is because maltose, which is a major component of wort and which is removed during fermentation, inhibits the gel formation of β-glucan in wort. Gel formation of beer β-glucans is effectively inhibited by urea, suggesting that hydrogen bonds are formed between β-glucan chains during gelation. Clarification of beer by centrifugation can also result in gel formation.

The fact that shear forces induce gel formation has suggested the following mechanism for barley β-glucan gels: Shear forces cause the molecules to orient in such a way that intermolecular bonds can form between long sequences of (1→4) linkages in adjacent molecules. Gel formation can also occur at low temperatures [185,186]. An increase of gel strength (studied by compression tests) with increased concentration was observed for barley β-glucans with molecular weights of 50,000 to 300,000 [182]. The gelling rate increased with decreased molecular weight. A similar trend has been observed for low-molecular-weight oat β-glucan [159]. Oat β-glucans with molecular weights of 35,000 to 140,000 form a gel. The lower the molecular weight, the higher the $G'$ and the faster the gelling. The stress and Hencky strain at failure increased with increased molecular weight.

The rheological properties of hydrolyzed β-glucan gels of oat have been studied by oscillatory shear measurements [19]. Gel formation of oat β-glucan occurs after a limited acid hydrolysis despite the slight decrease in molecular weight. The mechanical spectrum of nonhydrolyzed oat gum is typical of a solution: At low frequency, the loss modulus ($G''$) is higher than the storage ($G'$) modulus. In the case of the hydrolyzed gums, at low frequencies (0.01 to 1 rad/s), $G'$ is higher than $G''$, indicative of a gel. The chemical structures of nonhydrolyzed and hydrolyzed β-glucans are similar and cannot explain the marked differences in rheology. As possible explanation, Doublier and Wood [19] suggested that the lower molecular weight molecules are more mobile, and cellulose-like blocks in the molecules achieve the orientation necessary for aggregation. Over a longer period of time, this might also occur with nonhydrolyzed β-glucan, hence the reported tendency to gel on storage [11]. Lazaridou et al. [159] suggested that molecular size rather than fine
structure determines the gelling behavior. In their study, samples with the lowest proportion of long cellulosic-like chain segments showed the greatest tendency to gel.

5.3.2.5 Significance in the Malting and Brewing Process

Variatetal and environmental factors affect the level and structure of \((1\rightarrow 3), (1\rightarrow 4)\)-\(\beta\)-glucans in the barley endosperm cell wall. \(\beta\)-Glucan content is controlled by two or three genes [187,188], but the availability of moisture during grain ripening appears to have an effect, too, with dry conditions leading to high \(\beta\)-glucan levels [189–192] and rain causing a decrease in \((1\rightarrow 3),(1\rightarrow 4)\)-\(\beta\)-glucan content [190,193–195].

The extent to which \(\beta\)-glucan is degraded during malting depends on the moisture content of the steeped grain, on the amount of cell-wall-degrading enzymes, and on the amount and structure of the cell walls. During malting, the \(\beta\)-glucan of whole barley grain is degraded from an initial level of about 3.0 to 4.5% to about 0.2 to 1.0% [196–200]. The total \(\beta\)-glucan of sorghum and wheat grain does not decline during malting [34]. Malting of barley also causes a reduction in the molecular weight of barley \(\beta\)-glucan.

A barley with thin cell walls and able to synthesize high levels of \((1\rightarrow 3), (1\rightarrow 4)\)-\(\beta\)-glucanases might be expected to form well-modified malts with a high extract yield [2]; however, no link has been found between malting rate and cell-wall thickness. Large numbers of small cells seem to be more effective than thick cell walls in preventing enzymatic modification [201].

Breakdown of the endosperm cell walls initiates from the scutellum and advances more or less uniformly through the grain [202–204]. At the later stages of the malting process, the secretion of hydrolytic enzymes from the aleurone is accelerated. The different modification of cell walls could be explained by assuming that the enzymes produced by the two tissues, the scutellum and the aleurone, differ from each other both qualitatively and quantitatively [200].

Other germinating cereals also contain \(\beta\)-glucan-degrading enzymes [205–207]. The scutellum in rice [208,209], sorghum [207], wheat, rye, and oat [209] plays an important role in the secretion of both \((1\rightarrow 3),(1\rightarrow 4)\)-\(\beta\)-glucanase and \(\alpha\)-amylase [210]. In sorghum and wheat malts, only limited cell-wall degradation occurs during malting. Degradation of starch and protein takes place without extensive degradation of cell walls.

Optimization of the steeping program is important for uniform modification [211]. Low steeping temperatures of 13 to 16°C and a high steeping degree of 46% promote the production of cell-wall-degrading enzymes. External gibberellic acid, a germination accelerator, improves the modification rate [212].

Three processes involving \(\beta\)-glucans take place during mashing: extraction of the water-soluble fraction, dissolution of the insoluble fraction, and
enzymatic degradation of dissolved β-glucans [7,213,214]. While bound to proteins, β-glucans are insoluble. Endo-β-(1→3),(1→4)-glucanase is responsible for the degradation of the high-molecular-weight β-glucans, but the activity of this enzyme decreases significantly at 50°C. However, endo-β-(1→3)-glucanase and β-glucan solubilase, which are more thermostable, solubilize β-glucans from the intact cell walls throughout the mashing. β-Glucan solubilase is a carboxy-peptidase that degrades ester linkages between proteins and β-glucans [157]. Problems may appear if high-molecular-weight β-glucans are extracted during mashing at temperatures at which cell-wall-degrading enzymes are not active enough [215]. A high-molecular-weight β-glucan can be extracted from malted barley at 100°C but not at 75°C [34]. The temperature program used in mashing, grist particle size, mash thickness, and degree of mixing also influence the amount of β-glucans extracted [7].

Several problems in brewing may be caused by β-glucans, including: (1) decreased rates of wort separation and beer filtration, and (2) formation of hazes and gelatinous precipitates in the final beer. Yields of the extract will be reduced if filterability is poor or if the endosperm cell walls are not well modified and the starch and protein enclosed by the cell walls do not become accessible to amylases and proteases. At low temperatures, the rate of wort separation is decreased by the high viscosity of wort. A strong correlation has been found between the high molecular weight of β-glucans and beer filterability [124,214]. As well, β-glucan gels decrease beer filterability. β-Glucan molecules aggregate with each other and possibly with other large molecules to form colloidal suspensions. Hazes due to β-glucans alone are unknown [215]. Commonly, β-glucans are associated with proteins and polyphenols.

5.3.2.6 Significance in Animal Feeding

The highly viscous β-glucan in barley and oat limits the bioavailability of these grains. The bioavailability to chickens fed barley can be improved by enzyme supplementation, but not for pigs fed barley [138,216–218]. Enzyme supplementation has proved more effective for high-viscosity barleys such as Scout than for low-viscosity barleys such as Bedford [219]. A significant reduction in weight gain in chicks fed with hulless oats has recently been reported [220]. The gain was further reduced by the addition of oat gum. The effect of oat and barley β-glucan on chick growth cannot be entirely predicted on the basis of β-glucan concentration because contaminating microorganisms cause differences in endogenous β-glucanase activity [158].

5.4 CONCLUSIONS AND FUTURE

Cereal grains constitute a rich source of complex polysaccharides. In industrialized countries, increased consumption of nonstarch polysaccharides, with their many favorable physiological effects, is being recommended as a means...
to reduce the occurrence of diseases such as cancers of the breast and colon, coronary heart disease, and type 2 diabetes. In most industrialized countries, the main part of dietary fiber is derived from cereals but frequently in insufficient amounts [221]. Traditional rye-processing technology uses whole grains, with their high contents of minerals, vitamins, and fibers. As whole grains in general are nutritionally preferable to highly refined products, more efforts should be made to develop new bran-based products that are attractive to consumers in taste and texture, as well as nutritionally.

In the future, genetic engineering of crop plants will make it possible to produce varieties with improved functional properties. Genetic engineering of cereals has nevertheless proved difficult because of their general recalcitrance to the commonly used gene transfer techniques [222] and because of the genetic instability of transferred genes [223]. A method for barley has been developed [224], and the first application of this method will be to transfer a fungal thermostable β-glucanase gene [225]. This can be regarded as a major breakthrough in the bioengineering of malting barleys and may point the way to gene-transfer methods for cereals in general.

REFERENCES


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6 Gums and Hydrocolloids: Analytical Aspects

James N. BeMiller

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6.1 INTRODUCTION

With the exception of gelatin, food gums, which are also called hydrocolloids, are water-soluble or partially water-soluble polysaccharides. With the exception of xanthan, gellan, and curdlan, which are produced by microorganisms, polysaccharide food gums are obtained from land or marine plant sources. Food gums/hydrocolloids are often the determinants of texture, other quality attributes, stability, and applicable processing methods, even when naturally occurring and not added as ingredients.

Interest in analyzing food products for the various types of carbohydrates (e.g., digestible vs. nondigestible, metabolizable vs. nonmetabolizable, caloric...
Carbohydrates in Food

The very nature of polysaccharides presents analytical difficulties. They have a variety of chemical structures, shapes, solubilities, and molecular weights. In general, plant polysaccharides do not have repeating-unit structures. As a result, they are polymolecular; that is, their structures vary from polymer molecule to polymer molecule, with it being unlikely that any two molecules have exactly the same structure. In addition, average structures can, and do in most cases, vary with the cultivar grown, climate during the growing season, other environmental factors such as fertilization and application of herbicides, species, and, in some cases, processing conditions. Some food gums are neutral; some are anionic. Some are linear; some are branched but still effectively linear, and some have branch-on-branch, bush-like structures [1]. Some are composed only of sugar units; some are substituted with ether, ester, or cyclic acetal groups, either naturally or as a result of chemical modification. Some are completely soluble in cold water; some are completely soluble only in hot water. Some require aqueous solutions of acids, bases, or metal ion-chelating agents for dissolution and extraction from a food matrix. Some, such as cellulose, are insoluble in any food system. Whether from a plant or a microbial source, polysaccharides are polydisperse; that is, populations of their molecules exhibit a range of molecular weights rather than having a specific molecular weight. Often gum/hydrocolloid producers reduce the average molecular weights of their products in order to have available different viscosity grades; such depolymerization increases the polydispersity. Additional analytical difficulties result from the generally low concentrations (0.01 to 1%), the range of use levels in foods, and the increased use of blends of gums to extend functionalities.

These factors complicate determination of the types and amounts of polysaccharides in a food product. Most current methods, almost all of which have decided shortcomings, depend on extraction of the gums, followed by fractionation of the extract with concomitant losses of material. Sometimes, the gum is then hydrolyzed and identified through its constituent sugars. Under common acid-catalyzed hydrolysis conditions, however, constituent sugars are released from polysaccharides at different rates and are destroyed by hot acidic solutions at different rates; therefore, even determining the exact monosaccharide composition of polysaccharides is difficult and may not be achieved in some cases.

It might be assumed that the polysaccharides in food products will be known because of labeling laws and that the needs, therefore, are more quantitative than qualitative; however, adulteration of gums, especially locust bean gum and
gum arabic, is a problem that must be addressed by advanced analytical techniques. Also, fermentations may produce polysaccharides; for example, yogurt and frozen yogurt-type products may contain polysaccharides that function as stabilizers but which are not listed on an ingredient label because they have been produced during processing by microorganisms rather than added as ingredients. Identification of such an unknown polysaccharide is generally much more difficult than quantitative analysis of a gum for which the identity is known.

One approach for determination of most polysaccharide food gums in foods can be described but is generally not available. This approach is an application of the reporter unit concept — identification of a particular monosaccharide unit, functional group, or ratio of monosaccharide units that is unequivocally characteristic of a particular polysaccharide, ideally of each polysaccharide found in the food product. Better would be a method that could be applied to a whole food product without isolation and purification of the gums that will measure the amount of each gum or reporter unit quantitatively. Determination of acidic polysaccharides presents the greatest challenge. Glycosidic bonds of uronic acid units are difficult to hydrolyze and uronic acids decompose easily, so considerable destruction results under the more severe conditions required to release them. (The \( \text{L}- \) guluronic acid of alginates is released more rapidly than is the \( \text{D}- \) mannanuronic acid but also decomposes more readily, so, at the point where the degree of acid-catalyzed hydrolysis is at its optimum, any \( \text{L}- \) guluronic acid that was present may already be completely destroyed.) Carrageenans undergo acid-catalyzed hydrolysis more readily than do other food gums because of the 3,6-anhydro-\( \text{D}- \) galactosyl units, but these units are also destroyed in hot acid.

No single approach that will determine (either qualitatively or quantitatively) all gums/hydrocolloids in foods is available. The problems associated with the determination of gums in foods have been reviewed [2,3]. A review (with 95 references) describes the challenges and approaches taken in the quantitative determination of a single family of gums (the carrageenans) in food products [4]. This chapter cannot describe how to determine exactly the kinds and amounts of the various gums in food products for the reasons discussed above, only in general what has been and is being done and the problems and uncertainties associated with current methods. Only methods for the determination of gums other than those derived from a starch are presented. Analysis of starch-based gums (i.e., food starches and modified food starches) is discussed in Chapter 9. Also, the methods for dietary fiber, either soluble or insoluble, are not covered here, with the exception of \( \beta \)-glucan, although dietary fiber (other than the usual food gums) may be added to processed foods for textural as well as physiological functionality. Such analysis is discussed in Chapter 4. Because oligosaccharides are not considered to be food gums/hydrocolloids, their determination also is not covered, except for brief mentions of analyses of fructooligosaccharides.
Great progress has been made in the determination of chemical structures (complete analysis by nuclear magnetic resonance [NMR] techniques, mass spectrometric techniques, methylation analysis employing gas–liquid chromatography/mass spectrometry [GLC–MS], reductive cleavage analysis) and molecular structures (x-ray fiber diffraction analysis) to the point where these determinations are fairly routine. Conformational analysis of polysaccharides by molecular modeling is a rapidly advancing field; yet, a method for the quantitative determination of all possible food gums/hydrocolloids in food products is not currently available. There remains a need for precise, reliable methods of analysis of food products for the specific content of gums/hydrocolloids.

6.2 CRITERIA OF IDENTITY AND PURITY

Qualitative identification, specifications, and approved tests and analytical methods for all major gums/hydrocolloids, including modified starches used as food additives, and even minor ones, such as methylethylcellulose, have been published for the United States [5] and Europe [6] but are not specific in most cases. The *Official Methods of Analysis* of the Association of Official Analytical Chemists [7] contains methods for the analysis of agar in meat; alginates in chocolate products, chocolate frozen desserts, chocolate beverages, and food dressings; pectic acid in cacao and fruit products; and total gums in ice cream and frozen desserts, mayonnaise, French dressing, salad dressing, and soft curd cheese. All of these, too, are only inconclusive qualitative methods, and not all of the gums approved for food use are included. Of those methods that determine total gums, not all are applicable in the presence of starch, and some do not include certain gums.

In several cases, regulatory limits have been placed on nitrogen contents. That works well with gums such as xanthan, gellan, carrageenans, and alginates, which should contain only minimal levels of contaminating proteins and peptides. (Cellulose derivatives contain none.) It does not work well as a criterion of purity for gum arabic, which is a protein–polysaccharide, nor for gums such as guar and locust bean (carob) gums, which are seed endosperm flours. The allowance of alkali-treated seaweed flour (Philippine natural-grade carrageenan, processed Euchema seaweed, semirefined carrageenan) as carrageenan introduces an additional complication.

6.3 GUM ISOLATION AND FRACTIONATION

As already stated, most current methods for the identification and quantification of gums require their isolation and purification. This is necessary because of interfering substances, such as native or added starch (or modified starch products made by a variety of treatments), including the starch added as a
component of a cereal flour; proteins, which can associate with anionic polysaccharides and react with reducing sugars released by hydrolysis; and native cellulose, hemicelluloses, and pectic substances of plant materials, including flours. For efficient extraction, prior removal of fat and other lipid-soluble material that can limit water penetration is usually necessary. Consideration must be given to interactions with (or adsorption on) components other than proteins, the fact that gum blends (e.g., carrageenan–locust bean gum and xanthan–locust bean gum) are often used because of the specific interactions of the gums with each other, and the fact that anionic gums, such as the carrageenans, are often used because of their ability to complex with specific proteins. Major differences in solubility and use levels have already been mentioned. Use of gums or gum combinations in the form of small gel particles as fat replacers or mimetics may further complicate isolation from the food product. For these reasons, stoichiometric extraction of gums and hydrocolloids from food products is a major challenge.

Several isolation schemes have been proposed [2,8–14]. No system yet devised can be applied universally. Most have common features, such as an initial removal of lipid and lipid-soluble substances, dissolution of soluble substances, depolymerization of starch with thermostable $\alpha$-amylases, removal of protein by specific precipitation or by enzyme-catalyzed hydrolysis, and precipitation of polysaccharides, essentially the only remaining biopolymers; however, a specific scheme must be developed and verified for each food product. Verification can be achieved by recovery and quantitation of a known amount of a specific gum added to a product during its preparation.

Lipid and lipid-soluble substances are usually removed with polar solvents such as chloroform–methanol solutions (e.g., 95:5 v/v) [10] or dioxane [8,9]; hexane and diethyl ether have also been used. Dioxane is miscible with water and cannot be used with liquid products. A solution of potassium or sodium hydroxide in ethanol [11] has been used to extract fat (and some protein), especially from meat products, with concurrent dissociation of protein–polysaccharide complexes.

Starch polymers are broken down into soluble fragments by high-temperature digestion, often with a mixture of thermostable $\alpha$-amylase and glucoamylase (amyloglucosidase) [10,12]. (Use of resistant starch as dietary fiber adds a challenge to the removal of starch.) Low-molecular-weight, soluble fragments are then removed by dialysis, or the remaining biopolymers are precipitated with ethanol or acetone.

Protein is removed by digestion with papain or a bacterial alkaline protease [10,12] or by precipitation with trichloroacetic acid (TCA) [8,11] or sulfosalicylic acid [9,12]. Care must be taken with enzyme-catalyzed hydrolyses of proteins because commercial enzyme preparations are never devoid of carbohydrase (glycohydrodase) activities. Alkaline proteases are the proteases of choice because glycohydrodases have acidic pH optima. Problems can also be
encountered when using acid precipitation, because some gums, such as alg- 
inates and low-methoxyl pectins, also precipitate when their solutions are 
acidified.

Note: A procedure routinely employed in my laboratory for the removal of 
protein during the purification of water-soluble polysaccharides isolated from 
plant material — passage of a solution through a column of a strong cation-
exchange resin in the H⁺ form [15] — does not seem to have been employed 
in published reports of methods for the determination of gums in foods. Only 
proteins, not food gums, can be cationic, and proteins will have a net positive 
charge in the acidic solution produced by cation exchange; therefore, proteins 
(and only proteins, not neutral or anionic polysaccharides) will bind to the 
column. Polysaccharides, including all protein–polysaccharides examined to 
date [16], will pass through the column and can be recovered from the effluent.

Finally, the gums/hydrocolloids remaining in solution are precipitated by 
the addition of ethanol, acetone, or 2-propanol. Although this is a standard 
procedure whereby the water-soluble organic solvent is added slowly to a rapidly 
stirred solution of the gum, it is not without its problems. First, polysaccharides 
do not precipitate well from very dilute solutions, so concentration may be 
required. Generally, three volumes of 95% ethanol are added (final concentration 
= 71% ethanol v/v). This is usually sufficient for high-molecular-weight polysac-
charides; however, gums are available in a wide range of viscosity grades, 
including very-low-viscosity (very-low-molecular-weight) products for certain 
applications. These products may not precipitate well, even by addition of four 
volumes of ethanol (final concentration = 76% ethanol v/v). Precipitation of 
many polysaccharides becomes more thorough with the addition of a salt.

Separation may involve fractional precipitation [17] of a solution of 
extracted gums obtained by redissolution of the polysaccharide precipitate 
obtained by the addition of three to four volumes of ethanol. Most anionic 
polysaccharides can be made insoluble (i.e., precipitated) by their conversion 
into cetylpyridinium salts [18–21]. Some, such as alginates [22] and low-meth-
oxyl pectins [23], can be precipitated with calcium ions or by solution acidifi-
cation, although gelation may also occur. Gellan gum may be unique in being 
precipitated by monovalent cations [24], except for κ-type carrageenans, whose 
potassium salts are insoluble [25]; however, in both cases, gel particles result, 
or at best a gelatinous precipitate. A combination of forming cetylpyridinium 
salts and column chromatography has been used to separate neutral from acidic 
polysaccharides and acidic polysaccharides from each other [26].

6.4 QUALITATIVE IDENTIFICATION

A variety of tests has been proposed and is available for the identification of 
gums [2,5–7,19,20,27–34]. They involve precipitation with specific salts or 
dyes, color formation with specific reagents, or gel formation with specific

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reagents. Results of these tests are generally only rough indicators of the presence of a gum at best; however, some precipitation methods and some color-forming reactions have been made into semiquantitative methods.

Certainly, better methods of identification are needed. Much better qualitative analysis would be obtained by determination of reporter units; for example, the presence of both, and only, D-galactose and D-mannose in a gum would indicate a galactomannan (guar, locust bean, or tara gum). (The ratio would indicate which, and the total would indicate the amount.) But, because galactomannan preparations contain endosperm protein, methods of identifying the specific protein (to be described later) are better approaches. The presence of D-mannuronic acid would indicate an alginate. The presence of D-galacturonic acid would indicate a pectin. The presence of D-mannose and D-glucuronic acid in a 2:1 mole ratio indicates xanthan, as does usually the presence of pyruvic acid, although it may also be present in red algal polysaccharides [35]. (D-Glucose units are too ubiquitous to be used as reporter units.) More difficult are the cellulose derivatives and the exudate gums, although the fact that the latter are arabinogalactans, with some molecules being attached to protein molecules, could help. Here, again, identification of the specific protein component of gum arabic preparations by immunoassay (to be described later) is a better approach. Use of specific hydrolases (also to be described later) is another approach.

6.5 QUANTITATIVE ANALYSIS

6.5.1 CHEMICAL AND BIOCHEMICAL METHODS

6.5.1.1 Hydrolysis and Methanolysis

Methods employing acid-catalyzed hydrolysis followed by analysis of the mixture of released sugars have been employed widely and extensively. Here, again, this procedure will only identify a gum unequivocally if the gum contains a specific reporter unit not found in other potentially naturally occurring or added gums (e.g., D-mannuronic acid of alginates). Quantitative determination can be approached in this way only if the method gives complete hydrolysis of the polysaccharide and zero destruction of the reporter monosaccharide. These two criteria are probably never achieved together. In traditional acid-catalyzed hydrolysis, contaminating protein results in the destruction of released sugars through the Maillard reaction. Quantitative determination can also be accomplished only if the absolute percentage of the reporter unit in the polysaccharide is known — for example, the fact that, in gellan gum, D-glucuronic acid makes up about 27% (theoretical) and L-rhamnose about 23% of the monosaccharide products (after hydrolysis). (But, even here, the dry weight amount of the gum actually added to the preparation is a function of its acyl content and salt form.) Reporter unit amounts, such as D-mannuronic
acid in alginates, D-galacturonic acid in pectins, L-arabinose in gum arabic, and D-galactose (if no lactose is present) or D-mannose in galactomannans, can be used to calculate the amount of the gum present only if the percentage of that unit in the added gum is known, because percentages can vary with the source of the gum and sometimes with the processing parameters employed in its production. Ratios of constituents can also be used for qualitative and quantitative analysis, such as the ratio of D-mannose to D-glucuronic acid (2:1 mole/mole) in xanthan, but identification in this way may require a highly purified gum isolate to remove interfering sources of either monosaccharide unit, and purifications always result in some loss of the gum being determined. General, representative percentages of monosaccharide units in some common gums have been published [2,36]. Others are easily obtained [37,38].

The best conditions for conventional acid-catalyzed hydrolysis appear to be 4-M trifluoracetic acid at 121°C for 1 hour or at 100°C for 6 hours [39]. These conditions minimize problems associated with incomplete hydrolysis of uronic-acid-containing polysaccharides, decomposition of labile sugars, and losses associated with neutralization and work-up of the hydrolyzate. (TFA is removed by evaporation.)

Methanolysis has also been employed [9,36,39–43]. Methanolysis allows recovery of very labile sugars such as 3,6-anhydrogalactose; thus, it is useful for the identification of red algal polysaccharides such as agar and carrageenan [36,41] and also for uronic-acid-containing polysaccharides [42]. Its drawbacks are that it gives incomplete conversion of uronic-acid-containing polysaccharides into methyl glycosides and gives multiple products for each unit (i.e., methyl α- and β-glycosides of furanose and pyranose ring forms). For the latter reason, methanolysis may have to be followed by hydrolysis of the methyl glycosides. Methanolysis following an enzymic prehydrolysis has been used to determine both acidic and neutral sugars of cell-wall polysaccharides [43].

Reductive hydrolysis has been applied to agars and carrageenans in order to preserve the 3,6-anhydro-D-galactosyl units (i.e., to prevent their destruction) [44–46].

Solvolyis by liquid hydrogen fluoride followed by hydrolysis of the resulting glycosyl fluorides seems to address successfully the problem of complete conversion to monosaccharide units without their destruction, even conversion of uronic-acid-containing polysaccharides [47] and even in the presence of contaminating protein [48], but has not yet been applied to the determination of food gums. Handling of liquid hydrogen fluoride is a major drawback.

Hydrolysis may also be effected with enzymes. Because enzymes are substrate specific, this approach leads to both qualitative and quantitative analysis. Treatment of grains or products of grains containing (1→3),(1→4)-β-glucans with lichenase (a β-glucanase) yields fingerprint oligosaccharides
Likewise, treatment of pectins with pectolytic enzymes produces oligogalacturonic acid products, with different enzyme preparations and treatment procedures giving different product mixtures [50].

After conversion of a polysaccharide into its monosaccharide constituents, chromatographic methods are used for their identification and quantitation. High-performance liquid chromatography (HPLC) using anion-exchange (AE), amino-bonded, cation-exchange, and, more recently, reversed-phase [41,42,49] columns is the generally preferred approach. Refractive index, pulsed-amperometric detection (PAD), and ultraviolet (for uronic acids) detectors are used. A combination of high-performance, anion-exchange chromatography (HPAEC) and PAD is the most generally applicable and commonly used procedure (resulting in a large number of reports) but is not without its problems, most notably the fact that response factors vary from compound to compound and continually change over time. Because HPLC analysis evolved and progressed so rapidly and became the method of choice, reviews of mono- and oligosaccharide analysis by HPLC [51–54] quickly became dated and could not keep up with the plethora of reports and advancements. A literature search must be undertaken for each particular gum/hydrocolloid to be determined to find out which methods of extraction, methods of hydrolysis, HPLC columns, detectors, and conditions have been used successfully. The methods then should be adapted to the particular food product being analyzed.

Before HPLC, gas–liquid chromatography (GLC) [55] was used. It is still used to some extent [56] but requires several chemical steps to convert the sugars into volatile derivatives. For precision and accuracy, each step must be stoichiometric. That condition is, at least, closely met in most cases. The most common derivatives used are alditol acetates [57–59]. Other derivatives have also been used [60–64]. GLC has largely been replaced by HPLC for other than methylation analysis of polysaccharides.

Cellulose derivatives, such as carboxymethylcellulose, methylcellulose, hydroxypropylcellulose, and hydroxypropylmethylcellulose, are problematic in any analysis because of the large number of different monosaccharide units that may be present in a single product [65,66].

An alternative to chromatographic analysis is determination of specific reporter sugars using specific enzymes. Enzymic methods for the determination of L-arabinose [67], d-galactose [68], d-glucose [69–71], d-xylose [72], d-mannose [73], d-galacturonic acid [74], other monosaccharides, and alditols have been developed. Enzymic methods are easily automated, generally have high specificity for the sugar being determined, can measure very small amounts of the sugar, generally do not require high purity of the sample being analyzed, and do not require expensive equipment; however, no reports indicate that they have been extensively employed for the determination of gums and hydrocolloids in foods except in one or two cases, to be discussed in the next section and next paragraph.
Galactose oxidase has been used in a direct determination of galactomannan [75], but, for a quantitative determination, the mole percent of D-galactose in the galactomannan and the degree of oxidation must be known. Other substances containing D-galactose unsubstituted at O-6, such as lactose, would also give a positive result [68].

6.5.1.2 Enzymic Methods

Although specific enzyme-catalyzed hydrolysis has been widely and extensively employed for structural analysis and identification of glycoproteins, glycolipids, and proteoglycans, it has not been employed to any significant extent for the determination of polysaccharides, other than starch (Chapter 9), in foods. There are a few recent developments. Enzymes specific for depolymerization of chitin [76], galactomannans [77], mixed-linkage β-glucans [78], pullulan [79], xanthan [80], xylan [81], acetylxylan [81], arabinoxylan [81], cellulose and cellulose derivatives [82], hemicelluloses [83], and pectin [84] have been described. Certainly, other enzyme-catalyzed hydrolytic methods could be developed. The use of enzymes to release specific lytic products from polysaccharides allows the use of reporter units other than monosaccharides for identification and quantitation. A quantitative, spectrophotometric method based on specific enzyme-catalyzed hydrolysis has been established as an official method for mixed-linkage β-glucans [85–88]. This method is the only official, specific, quantitative method for a polysaccharide in a food product. The method involves treatment of the grain or food product with a β-glucanase that converts the mixed-linkage β-glucan into oligosaccharides. Addition of β-glucosidase converts the oligosaccharides into D-glucose, which is determined with a coupled-enzyme glucose oxidase–peroxidase reagent [85–88]. Alternatively, the released D-glucose can be measured with a glucose electrode [89]. Recombinant endopectate lyase has been used for identification of pectin [90].

Inulin has long been used for the determination of renal glomerular clearance. This application requires analysis of blood plasma and urine for the polysaccharide. Coupled-enzyme assays involving inulinase and a method to measure the released D-fructose have been developed [91,92], but there is no evidence of this procedure being applied to food products.

Because enzymes are specific in terms of both substrate and products, enzymic methods could easily become the method of choice for at least the qualitative, if not quantitative, analysis of foods; for example, a simple reduction of viscosity upon incubation with a specific, purified enzyme could provide rapid and essentially unequivocal identification or confirmation of the presence of a particular gum or family of gums (e.g., galactomannan). Care must be taken, however, to use highly purified enzymes because most commercial preparations contain more than one glycohydrolase activity. Surely, in some cases, coupled-enzyme methods could be developed that involve the
release of a specific unit followed by enzymic determination of that specific unit, whether a monosaccharide or an oligosaccharide — both either unmodified or modified (e.g., as a 4,5-ene from the action of a lyase) — or a substituent group. Chemical, enzymic, and instrumental [93] methods, for example, have been described for the detection and determination of pyruvic acid from xanthan.

### 6.5.1.3 Colorimetric/Spectrophotometric Methods

Colorimetric methods are among the oldest methods used for the analysis of carbohydrates, and they persist because of ease of use. Total carbohydrate can be determined, using D-glucose as a standard, by colorimetric methods [33,94–98]; however, these determinations, while valuable, are inexact even for polymers that contain only D-glucose, such as cellulose, β-glucans, and the starch polysaccharides, because of differences from polysaccharide to polysaccharide in monosaccharide release and differences from sugar to sugar in their destruction and in the formation of the colored compounds from them. Specific colorimetric methods have been applied to the quantitative and qualitative determination of uronic acids [29–31], 3,6-anhydrogalactose (carrageenan) [34,99], pyruvic acid (xanthan) [93], pectins [100–102], alginates [103], gellan [104], and carboxymethylcellulose [32,105,106]. Because of interferences, none of these methods is appropriate for the direct analysis of a food product.

Methods other than the classic colorimetric/spectrophotometric methods involve measuring uronic-acid-containing polysaccharides after they are converted into salts with a fluorescent-, visible-, or ultraviolet-absorbing cationic dye [107–112] or via spectrophotometric titration with a cationic dye [113]. A flow-injection technique for the determination of β-glucan is based on its formation of a complex with Calcofluor [114,115]; however, these methods are sensitive to the degree of sulfation and therefore give different responses for the different red algal polysaccharides [4].

### 6.5.2 Chromatographic Methods

#### 6.5.2.1 High-Performance Liquid Chromatography

High-performance liquid chromatography (HPLC) methods have been developed for the determination of inulin in blood plasma and urine [116], pectin [117], and hydroxypropylmethylcellulose [118] and for the separation of oligosaccharides from inulin, other fructans [119–122], pullulan, xylan, and mannan [117]. In general, it seems to be most useful for chromatography of oligosaccharides and relatively low-molecular-weight polysaccharides. (Determination of oligosaccharides other than those related to inulin is not covered in this chapter.)
6.5.2.2 Size-Exclusion Chromatography

Size-exclusion (gel-permeation, steric-exclusion, molecular-sieve) chromatography (SEC) has not found much use (even when coupled to a multiangle laser-light-scattering [MALLS] detector) because of the polydispersity of the gums being determined, because several gums are available in a wide range of average molecular weights, because of the potential overlap in molecular weights of the gums being determined, and because of the potential of many gum molecules to associate with each other and with other biopolymers. Nevertheless, methods are available and have been used for the analysis of pectin [117,123], for the determination of carrageenan in food products [124], and for the comparison of gum arabics [125].

6.5.2.3 Capillary Electrophoresis

Capillary electrophoresis (CE) has been used to separate and measure acidic (i.e., charged) food gums [126], such as pectins [127] and carrageenans [128,129]. Because galactomannan gums are powdered endosperm tissue, they contain, in addition to a polysaccharide, endosperm proteins, cell-wall constituents, lipids, and ash. They can be identified by their constituent proteins. CE has been used to obtain protein profiles of guar gum and locust bean gum [130–133] and to determine whether locust bean gum has been adulterated with guar gum [131–133]. Such adulteration has become a major problem, and this approach seems to be a good one. Another good one is presented in Section 6.5.4.

6.5.3 Physical Methods

6.5.3.1 Infrared Spectroscopy

An active area of investigation in recent years has been that of Fourier transform infrared spectroscopy (FTIR) to identify and quantify food gums. These methods are simple and rapid, but minimal percentages in the food required for detection are somewhat greater than those required for some other methods. Spectral libraries have been compiled for a range of food gums [134], including κ-, ι-, and λ-type carrageenans [135–140], pectin (polygalacturonic acid, amidated and nonamidated pectins) [137,138,141], galactomannans [137], alginates [137], exudate gums [137], xanthan [137], gellan [137], and cellulose derivatives [142]. Also examined as analytical tools have been near-infrared reflectance (NIR) spectroscopy [142–144] and Raman spectroscopy [142,145].

6.5.4 Other Methods

Polyacrylamide gel electrophoresis (PAGE) has been applied to the analysis of marine algal polysaccharides [146] and for the authentication and identification
of gum arabic [147]. Isoelectric focusing has also been applied to the latter [147]. Because gum arabics contain a protein component as part of their structure, antibodies can be used to determine the source species and to authenticate the preparation [148,149]. Likewise, because galactomannan gums are powdered endosperm tissue and contain protein, antibodies can be used to detect them in mixtures [150]. Immunosorbent assays have also been developed for κ-carrageenan [151–153], which does not contain protein as part of its structure. The presence of any cross-reactivities in any of these methods is unknown.

Polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) analysis will detect guar gum in locust bean gum in amounts as low as 1% [154,155]. High-performance thin-layer chromatography (HPTLC) has been applied for the quantitative determination of inulin in food products [156]. Proton nuclear magnetic resonance will give a quantitative measure of κ-, ι-, and λ-type carrageenans in mixtures of them [157–158]. 13C-NMR will also reveal the composition of carrageenan blends [159], and carrageenan has also been determined by potentiometric titration using polymeric matrix tubular membrane polyion sensors [160].

Matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) is useful for determining the distributions of oligosaccharides, easily up to DP 20 and as high as DP 50, such as those in malto- or inulodextrins or inulin preparations themselves [161–164], but analysis of oligosaccharides is not within the scope of this chapter. Although coupling of MALDI-MS with time-of-flight mass spectrometry (MALDI-TOF-MS) allows a wider mass range to be determined [165], this technique is not useful for the determination of food gums in food products for the same reason that SEC is not. Mass spectrometry coupled to capillary liquid chromatography and electrophoresis [166–168] may find use in polysaccharide analysis.

REFERENCES


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7 Gums and Hydrocolloids: Functional Aspects

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7.1 INTRODUCTION

With the noticeable exception of starch, which plays two roles (first, as a major nutritional ingredient and, second, as a texture agent), the use of polysaccharides...
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by the food industry is primarily based on the only role they play in the texture of foodstuffs. For this reason, they are generally classified as texture agents, in addition to a second class of such ingredients, the emulsifiers. Due to this important role in the quality of processed foodstuffs, these food ingredients have been investigated to a large extent by many scientific groups.

Food materials can be regarded as multiphasic systems [1,2]. In many cases, the aqueous phase is continuous while the dispersed phase is composed of lipid droplets or solid particles as fibers, protein aggregates, crystals, or gas bubbles. Such complexity does not allow one to define these systems merely as emulsions, dispersions, or foams (see Table 7.1). Biopolymers by themselves should be considered in the aqueous medium as colloidal materials, due to their large size as macromolecular entities when compared to the size of water molecules. This is why these macromolecules are classically defined as hydrocolloids. Ice cream is a good example of such a complex colloidal system. It is a foam (air content approximately 50% by volume), the walls of which are a frozen aqueous dispersion containing ice crystals, fat droplets from milk (themselves crystallized), casein micelles, and other solid particles (e.g., cocoa) [3]. Adding a polysaccharide, such as locust bean gum, guar gum, κ-carrageenan, or pectins, allows one to control the texture of the end product. The hydrocolloid plays a role during the process by controlling the rheology of the continuous phase (in stabilization of the mix as well as during incorporation of air at the freezing stage) and by its involvement in the control of ice crystal growth.

### TABLE 7.1

**Food Products as Multiphasic Systems**

<table>
<thead>
<tr>
<th>Continuous Phase</th>
<th>Dispersed Elements</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aqueous phase</td>
<td>Macromolecules, casein micelles, fat globules</td>
<td>Dairy products, yogurt, cheese and analogs, milk gels, dairy desserts, whipped cream, ice cream</td>
</tr>
<tr>
<td>Lipid droplets</td>
<td>Mayonnaise, salad dressings</td>
<td></td>
</tr>
<tr>
<td>Starch granules</td>
<td>Sauces, soups, cake batter</td>
<td></td>
</tr>
<tr>
<td>Fibers, membranes</td>
<td>Beverages, jams, fruit preparations, processed meat products, cereals</td>
<td></td>
</tr>
<tr>
<td>Crystals (ice, triglycerides, sugars)</td>
<td>Ice cream, whipped cream</td>
<td></td>
</tr>
<tr>
<td>Gas bubbles</td>
<td>Whipped cream, mousses, baked products</td>
<td></td>
</tr>
<tr>
<td>Lipid phase</td>
<td>Water droplets</td>
<td>Butter</td>
</tr>
<tr>
<td>Triglyceride crystals</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sugar crystals</td>
<td>Chocolate</td>
<td></td>
</tr>
<tr>
<td>Plant particles</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The functional properties of polysaccharides are generally considered with regard to the results that are expected (Table 7.2). In the simplest situations, functionality is the straightforward result of the properties of the biopolymer in an aqueous medium (i.e., its thickening or gelling properties). In many cases, however, when more complex properties have to be analyzed, the link between the behavior of the biopolymer and the expected property is less clear. This is the case, for example, for binding properties. Also, many times several mechanisms are involved. Emulsions and suspensions are good examples of such a complexity. These systems can be stabilized either through control of the rheology of the continuous phase (e.g., by enhancing its viscosity) or through control of interfacial properties. The mechanism to be monitored (i.e., creaming, flocculation, or coalescence) will determine the method for stabilizing the emulsion.

The functional properties of hydrocolloids lie mostly in the physicochemical mechanisms underlying their behavior in an aqueous medium. In other words, they are the result of macromolecule–water and macromolecule–macromolecule interactions, as well as interactions of macromolecular chains with the surface of the dispersed entities (solid particles, droplets, or gas cells) (see Table 7.3).

All of these mechanisms are driven by the thermodynamics of the system. Water solubility is related to solvent quality — that is, the strength of interactions between the polysaccharide and water (the solvent) through hydrogen bonds created by means of hydrophilic groups along the macromolecular chain. Hydrodynamic volume and thickening properties are related to water–polysaccharide interactions. In contrast, gelation takes place as a result of a subtle equilibrium between polymer–polymer and polymer–solvent interactions.
TABLE 7.3
Main Physicochemical Mechanisms Involved in the Function of Hydrocolloids

<table>
<thead>
<tr>
<th>Physicochemical Property</th>
<th>Functional Property</th>
</tr>
</thead>
<tbody>
<tr>
<td>Macromolecule/solvent interactions</td>
<td>Solubility, swelling</td>
</tr>
<tr>
<td></td>
<td>Viscosity increase, thickening</td>
</tr>
<tr>
<td></td>
<td>Binding</td>
</tr>
<tr>
<td></td>
<td>Stabilization</td>
</tr>
<tr>
<td>Macromolecule/macromolecule interactions</td>
<td>Gelling</td>
</tr>
<tr>
<td></td>
<td>Binding</td>
</tr>
<tr>
<td></td>
<td>Stabilization</td>
</tr>
<tr>
<td>Surfactant or macromolecule interactions with:</td>
<td>Adsorption, emulsification</td>
</tr>
<tr>
<td>Oil droplets</td>
<td>Whipping, stabilization</td>
</tr>
<tr>
<td>Solid particles</td>
<td></td>
</tr>
<tr>
<td>Gas</td>
<td></td>
</tr>
</tbody>
</table>

Junction zones have to be created in order to yield the three-dimensional network that gives the solid-like character of the system despite its high water content. Thermodynamics is also at the basis of the peculiar properties of mixed biopolymer systems. Phase separation takes place if polymers are incompatible. The incompatibility should result in phase separation and should yield two separated phases at thermodynamic equilibrium; however, additional phenomena can take place, particularly when one or two components can form physical gels. This impedes the phase separation to be completed at the macroscopic level, and the final structure of the system is not at thermodynamic equilibrium. The result is a complex morphology yielding a specific texture, whether pleasant or unpleasant, for the consumer. Molecular binding between unlike polymers has been suggested as a possible mechanism underlying the properties of mixed polysaccharide systems resulting in dramatic synergistic effects. These phenomena are not yet clearly understood, however, and the molecular mechanisms of binary biopolymer systems are still a matter of debate.

In the case of dispersed systems, adsorption of the polymer onto the interface may be desired. This phenomenon can be the major mechanism responsible for the stabilizing effect. Water–solvent interactions are also generally involved. When part of the macromolecular chain cannot interact with water, due to the presence of numerous hydrophobic groups, adsorption onto the interface is favored. In contrast, if the overall polymer chain is hydrophilic, there will be no affinity between the polymer and the interface. In such a case, the polymer is excluded from the vicinity of the particle, and a depletion–flocculation phenomenon may occur [4–6]. Particles are led to flocculate in order to organize themselves and hence to minimize the overall excluded volume.
In every situation, the rheological properties of the system are deeply changed when polysaccharides are present; therefore, rheological methods are the appropriate tools for studying the functional properties of hydrocolloids, as they allow one to characterize the overall properties of the investigated system. These can be directly related to the expected texture. Other properties, such as pourability, spreadability, and stabilization, can also be estimated in this way, but indirectly.

Another interesting aspect of rheological measurements lies in the fact that these methods provide direct information on the molecular mechanisms underlying the properties. These mechanisms can be related to entanglement of macromolecular chains, the lifetime of interchain interactions, or the density of junction zones. An accurate description of the functional properties exhibited by polysaccharides in real foodstuffs, as discussed above, would require many details that cannot be discussed here. The reader is referred to the extensive literature available on this topic [7–10]. Volumes from the series of Gums and Stabilisers for the Food Industry conferences held in Wrexham every 2 years also contain much discussion regarding the role of hydrocolloids in the fabrication of food products. This chapter primarily deals with the basic properties of hydrocolloids — water solubility, thickening properties, and gelling.

The thickening properties of food polysaccharides are classically evaluated by viscometric measurements. Before the advent of relatively low-cost rheometers around 15 years ago, gels were most often estimated using compression tests in order to arrive at the rigidity modulus. Of course, this requires the gels to be self-supporting, and weak gels cannot be characterized in such a way. In all cases, the rheological information remains quite limited; moreover, many intermediate systems in between these two types of systems that cannot be referred to as true macromolecular solutions or true gels are not easily characterized by these means.

The recent development of new and relatively inexpensive rheometers, either controlled stress or controlled strain, together with the capabilities of newer computers have yielded outstanding improvements in the characterization of macromolecular systems by means of viscoelastic measurements. As a result of this development in rheological instrumentation, the list of recently published studies of polysaccharides by rheological means is rather long and cannot be described in detail here. Instead, we have chosen to take typical examples from the literature in order to illustrate the application of rheological techniques for the understanding of the behavior of polysaccharide systems. Of the three types of methods available that depend on the way the deformation (or the stress) is imposed — oscillatory shear measurements, relaxation tests, and creep-recovery tests — the first method is the one employed most often, as it is easier to perform. Each system (polysaccharide alone or mixture) will result in a given time (or frequency) dependency of rheological parameters.
which will reflect a specific organization of macromolecules in the medium. This allows discussion of the results on the basis of the molecular mechanisms underlying the properties.

### 7.2 Behavior of Polysaccharides in Aqueous Solution

#### 7.2.1 Solubilization: A Critical Step

Solubilization is an important step in the use of hydrocolloids. The objective is to ensure that each macromolecule has been individualized in this process in order to develop fully the functional properties. This is illustrated in Figure 7.1.

**Figure 7.1** Schematic description of the different steps of the solubilization process of polysaccharides.
In practice, the solubilization will be achieved in two steps. The first requires a careful dispersion of the powder in order to prevent the formation of lumps. This is a critical point, because lumps are nearly impossible to solubilize. This requires individualization of each grain of the powder. Of course, this individualization will depend on the nature of the hydrocolloid, the size and porosity of the grains, the stirring conditions, and other variables. Many techniques are involved in fulfilling these conditions. As a rule, water uptake should be relatively slow and agitation as strong as possible. The less easily soluble polysaccharides are the more hydrophilic ones, for which the formation of lumps is difficult to avoid. The second step of the solubilization process aims at the complete individualization of the macromolecules, which must be achieved for thickening systems as well as for gelling ones. Water must penetrate into swollen particles and compete with interactions between macromolecules. A variety of outcomes can be encountered, depending on the strength of these interactions. In all cases, agitation and time are required.

Certain polysaccharides are solubilized quite easily in cold water because macromolecular interactions are relatively weak. This is the case with guar gum, xanthan gum, sodium alginate, pectins, and λ-carrageenan. Others, such as ι-carrageenan, κ-carrageenan, and locust bean gum, require heating for full solubilization to be ensured. In the case of carrageenans, heating induces the chains to adopt a disordered conformation that is a condition for these macromolecules to be water soluble. In the case of locust bean gum, heating breaks down the chain–chain interactions through the smooth zone of the mannann chains. Moreover, solubility can become more difficult to achieve when one is dealing with other aqueous media, such as high-salt solutions (brines), calcium-rich solutions (hard water, milk), or high-sugar solutions (sugar syrups).

### 7.2.2 Polysaccharides in Solution: Their Characterization

#### 7.2.2.1 In Dilute Solution: Intrinsic Viscosity

When the polysaccharide has been solubilized in water, viscosity measurements in dilute conditions provide an easy and interesting means to characterize the behavior of the polysaccharide in aqueous solution. If the concentration is low enough to yield a Newtonian behavior, determination of the intrinsic viscosity through extrapolation to zero concentration provides an indirect estimate of the molecular size of the macromolecule. An example is given in Figure 7.2 for a locust bean gum sample. From the viscosity value, generally obtained using a capillary viscometer, the relative viscosity is given by:

\[
\eta_{rel} = \frac{\eta}{\eta_c}
\]
where \( \eta \) is the viscosity of the solution, and \( \eta_s \) is the viscosity of the solvent. From the relative viscosity, the specific viscosity (\( \eta_{sp} \)) can be defined as:

\[
\eta_{sp} = \eta_{rel} - 1
\]

and the reduced viscosity as:

\[
\eta_{red} = \eta_{sp} / c
\]

where \( c \) is the polysaccharide concentration (expressed in g/dl or in g/ml). By definition, the intrinsic viscosity \( [\eta] \) is given by:

\[
[\eta] = \lim_{c \to 0} \eta_{sp} / c
\]

This parameter corresponds to the volume occupied by the mass unit in infinite dilute conditions. It is directly related to the molecular weight of the macromolecule.

The example given in Figure 7.2 for locust bean gum sample illustrates a double plot deduced from viscosity determinations at polysaccharide concentrations such that \( \eta_{rel} \) values were lower than approximately 2. Curve 1 is the plot of \( \eta_{red} \) and curve 2 is the plot of \( (\ln \eta_{rel})/c \) as a function of concentration. The straight lines that were obtained can be described by Huggins’ equation.
\[ \eta_{sp}/c = [\eta] + k[\eta]^2 c \]

and by Kraemer's equation:

\[ (\ln \eta_{\infty})/c = [\eta] + k''[\eta]^2 c \]

where \( k' \) is the Huggins coefficient, \( k'' \) is the Kraemer coefficient, and \( [\eta] \) is the intrinsic viscosity (in dl/g or in ml/g). It is clear that \( [\eta] \) is obtained from both plots by extrapolation to infinite dilution. The two plots must converge on the \( y \)-axis at the point corresponding to \( [\eta] \). It is also required that:

\[ k' - k'' = 0.5 \]

The value of the Huggins coefficient should lie between 0.3 and 0.8. Values higher than unity indicate that aggregation is likely to occur. In this example, we found that \( [\eta] = 14.5 \) dL/g, \( k' = 0.45 \), and \( k'' = -0.057 \), thus verifying the aforementioned principles. Such a procedure is valid without restriction when dealing with neutral polysaccharides such as galactomannans. For polyelectrolytes such as pectins, alginates, or carrageenans, several precautions have to be taken. In an aqueous medium, if no salt is added, the reduced viscosity increases as the polysaccharide concentration decreases (curve 1 in Figure 7.3). This is typical behavior of polyelectrolytes and is ascribed to a variation in the degree of coil expansion due to stronger intramolecular electrostatic repulsions as the polymer concentration decreases. In the absence of added salt, the ionic strength of the medium originates from the only polyelectrolyte, and as the concentration decreases the ionic strength decreases. As a result, the intramolecular repulsions are reinforced and the hydrodynamic volume of the macromolecule increases. This variation results in an increase of the reduced viscosity, making the extrapolation to infinite dilution no longer valid. A way to overcome this effect is by maintaining a constant ionic strength upon dilution. This can be achieved by using isoionic dilutions in which the decrease in ionic strength is compensated for by the addition of the salt; hence, the total ionic strength \( (I_t) \) is kept constant according to the relation:

\[ I_t = I_s + \lambda C_p \]

where \( I_t \) is the total ionic strength, \( I_s \) is the ionic strength arising from the added electrolyte, \( \lambda \) is the osmotic coefficient, and \( C_p \) is the polyelectrolyte concentration (in eq/l). Using such a procedure ensures that the normal Huggins equation is obeyed, as illustrated in Figure 7.3 (curves 2 to 6). Furthermore, if the electrolyte content is high enough so the second term of the above
relationship can be neglected, then the use of this procedure is not required, because \( I \gg \lambda C_p \) and \( I = I_c \). This is illustrated by curves 7 to 9 in Figure 7.3.

Variations of \([\eta]\) with ionic strength are related to the flexibility of the macromolecular chain and, at a relatively high ionic strength (typically, \( I > 10^{-2} \)), these can be expressed as:

\[
[\eta] = [\eta]_0 + S I^{-3/2}
\]

where \([\eta]_0\) is the intrinsic viscosity extrapolated to infinite ionic strength, and \( S \) is an adjustable parameter related to the flexibility of the polyelectrolyte as well as the molecular weight. Another parameter that does not take into account the effect of molecular weight can be estimated from:

\[
B = (S/[\eta]_{0,1})^{1/3}
\]
where \([\eta]_{0.1}\) is the intrinsic viscosity at \(I = 0.1\ M\), and \(B\), defined as an intrinsic flexibility parameter, characterizes the stiffness of the polyelectrolyte; the lower the \(B\) value, the higher the stiffness of the chain. A few values from the literature are given in Table 7.4 as an illustration. \([\eta]_{\infty}\) also has a specific meaning — it corresponds to the lower limiting hydrodynamic volume the polyelectrolyte can adopt and is related to dimensions of the unperturbed macromolecular coil.

For every polymer–solvent system, the intrinsic viscosity is directly related to the molecular weight according to the Mark–Houwink relationship:

\[
[\eta] = K\overline{M}_v^\alpha
\]

where \(\alpha\) is the Mark–Houwink exponent, and \(\overline{M}_v\) is the viscosity-average molecular weight. \(K\) and \(\alpha\) are related to the degree of molecular expansion and hence depend on the local stiffness of the polymer backbone and polymer–solvent interactions: \(\alpha\) varies from 0.8 (good solvent) to 0.5 (\(\Theta\) solvent, unperturbed dimensions) for coil-like polymers but can be as high as 1.8 for rod-like polymers. Typical values of \(K\) and \(\alpha\) are given in Table 7.5. Of course,
when one is dealing with polyelectrolytes, these parameters are strongly dependent on the medium conditions (pH, ionic strength). In practice, \([\eta]\) is directly related to coil dimensions according to the Flory–Fox equation, expressed as:

\[
[\eta] = 6 \Phi^{\frac{3}{2}} \frac{R_g^3}{\bar{M}}
\]

where \(\Phi\) is a universal constant, the Flory coefficient \((\Phi = 2.6 \times 10^{23} \text{ kg}^{-1})\), and \(R_g\) is the radius of gyration of the polymer coil. It infers that \([\eta]\) is directly related to the dimension of the coil and can be taken as an indirect estimate of its hydrodynamic volume.

These theoretical descriptions apply only to macromolecular systems exhibiting the behavior of a random coil. This is valid for the majority of polysaccharides but not for some of them displaying a very high stiffness, particularly xanthan gum. This polysaccharide is known to adopt a helical or coil conformation depending on temperature and ionic strength. It exhibits a helix-to-coil transition at a temperature around 40 to 50°C in distilled water, and this transition temperature increases with increasing ionic strength; therefore, at a normal temperature, and whatever the ionic strength, the conformation is a helix and can be regarded as relatively stiff or semirigid. As a result, the hydrodynamic volume adopted by such a rigid macromolecule in an aqueous medium is much higher than by a random coil. This can be illustrated by a comparison of \([\eta]\) values of guar gum to xanthan gum at equivalent molecular weight: for \(\bar{M}_r = 10^6\), \([\eta]\) would be 24 dl/g for xanthan gum compared to 8.3 dl/g for guar gum (Table 7.5).

As described below, knowledge of the behavior of polysaccharides in dilute aqueous solutions is a prerequisite to understanding the functional properties of these macromolecules. Details on the physicochemical basis of these behaviors and particularly on the relationships between intrinsic viscosity and macromolecular characteristics cannot be provided in this chapter; further information can be found in the literature [21–24].

### 7.2.2.2 Flow Behavior

#### 7.2.2.2.1 General Behavior

Determination of the intrinsic viscosity requires measurements to be performed in the dilute regime, typically \(c[\eta] < 1\), and, in these conditions, the behavior is Newtonian. Beyond this concentration, the shear rate dependence of the solutions has to be taken into account. Figure 7.4 shows the variations in shear stress as a function of shear rate, in linear coordinates, for two locust bean gum solutions (0.5% and 0.75%, for which \(c[\eta] = 6.0\) and \(9.0\), respectively). These concentrations lie well beyond the limits of the dilute regime. The behavior clearly is shear thinning, with no shear dependency; the “down” curve obtained with decreasing shear rate is superimposed over the “up”
Variations in apparent viscosity as a function of shear rate (logarithmic scale) are shown in Figure 7.5 for four concentrations. The shear-thinning behavior can be clearly seen. Moreover, a plateau defining a limiting Newtonian viscosity ($\eta_0$) is reached within the low shear rate range. It is only beyond a critical shear rate ($\dot{\gamma}_c$) that the behavior is shear thinning, the $\dot{\gamma}_c$

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value decreasing as the concentration increases. These flow curves can be described using empirical equations. One of the more popular is the Cross equation [21,23–26]:

\[ \eta_a = \eta_\infty - \frac{\eta_0 - \eta_\infty}{1 + (\tau \dot{\gamma})^m} \]

where \( \eta_a \) is the apparent viscosity at a given shear rate \( \dot{\gamma} \), \( \eta_\infty \) is the limiting Newtonian viscosity at a high shear rate, \( \eta_0 \) is the limiting Newtonian viscosity at a low shear rate, \( \tau \) is a structural relaxation time, and \( m \) is an exponent related to the shear-thinning behavior. The fits of the experimental results given in Figure 7.5 are illustrated by the continuous lines. In these examples, \( \eta_\infty \) was neglected because it is much lower than \( \eta_0 \). The equation can therefore be simplified to:

\[ \eta_a = \frac{\eta_0 - \eta_\infty}{1 + (\tau \dot{\gamma})^m} \]

Such a behavior is known to be typical of macromolecular solutions and has been reported for galactomannans as well as for other nongelling polysaccharides (e.g., dextrans, \( \lambda \)-carrageenans, cellulose derivatives) [27]. It has also been shown that these flow curves can be superimposed using \( \eta/\eta_0 \) and \( \dot{\gamma}/\dot{\gamma}_{0.1} \) as reduced variables with \( \dot{\gamma}_{0.1} \) corresponding to the shear rate at which \( \eta_a = \eta_0/10 \) [27]. Using such an approach, flow curves from different polysaccharides (guar gum, locust bean gum, alginate, \( \lambda \)-carrageenan, and hyaluronate) could be superimposed on a curve that could be expressed by the generalized Cross equation where \( m = 0.76 \) and \( \tau = 18/\dot{\gamma}_{0.1} \) [28].

It has been shown that \( \eta_0 \), depends on concentration and the intrinsic viscosity, \( [\eta] \). This is illustrated in Figure 7.6 for different guar gum samples for which variations of \( \eta_{\text{pol}} \), where \( \eta_{\text{pol}} = (\eta_0 - \eta_{\text{solv}})/\eta_{\text{solv}} \), as a function of the reduced concentration \( c[\eta] \), are shown. The three straight lines with slopes of 1.3, ~2.5, and ~5 define three concentration domains, the limits of which correspond to two critical concentrations \( c^* \) and \( c^{**} \), with \( c^*[\eta] \) close to 1 and \( c^{**}[\eta] \sim 6 \). Here, again, such a dependency is typical of macromolecular solutions. These two critical concentrations have a physical significance [30], in that \( c^* \) represents the upper limit of the dilute regime and is related to the onset of significant coil overlap; above \( c^* \), interpenetration of the macromolecular chains takes place. Meanwhile, a contraction of the coils progressively brings the chains to their unperturbed dimensions and, beyond the second critical concentration, \( c^{**} \), a concentrated regime is defined as the region where the coil dimensions become independent of concentration. Only beyond \( c^{**} \) (\( c^{**} = 0.4 – 0.5\% \) for the sample in Figure 7.5) is the thickener really efficient.
The broadness of the intermediate region as defined by \( c^* < c < c^{**} \) has been shown to depend on the compression possibilities of the coil, itself related to the polymer–solvent interactions; the better the solvent for the polysaccharide, the broader this intermediate domain [30–32].

Recently, several detailed investigations have been performed regarding the characterization of exopolysaccharides (EPSs) excreted by different types of food microorganisms [33–36], in light of the fact that, because the microorganisms are food grade, the EPSs may themselves be regarded as food ingredients rather than additives. As a general rule and despite a broad range of chemical structures, because these polysaccharides are linear, neutral, or polyelectrolytes, they display flow properties that are reminiscent of the flow behavior of food polysaccharides as described earlier: shear-thinning behavior, absence of thixotropy, and similar concentration dependency. Attempts to describe these flow behaviors on the basis of current theories of macromolecular solutions have failed, however, likely due to difficulties in obtaining very pure polysaccharides [33].

\[ \eta_{sp} \text{ as a function of } c[\eta] \text{ for five guar gum samples differing in their molecular weight. (These data were published initially in Robinson et al. [18] and have been replotted in Ross-Murphy [29] to show } c^* \text{ and } c^{**}. \]
7.2.2.2 Xanthan Gum: A Peculiar Case?

Xanthan gum is known to exhibit peculiar flow properties with pronounced shear-thinning behavior. At a very low concentration, typically 0.1% and below, this polysaccharide displays a flow behavior typical of a macromolecular solution, as described above; however, when this system is more concentrated it is very often reported to display a yield stress — that is, a critical stress above which flow is observed and below which the system can be regarded as solid-like. A flow curve is shown in Figure 7.7 for a 0.8% xanthan solution [37]. This curve has been plotted using data from two different methods. In the first one, classical viscosity measurements have been performed by increasing the shear rate step by step from about 10–2 to 640 s–1. In the second one, with γ ranging from about 2.5 × 10–4 to 10–2 s–1, the data have been obtained from creep experiments in the steady regime (i.e., at long timescales). This type of measurement is described below. In the first range, the flow curve could be described using a power law equation (\( \eta_a = K\dot{\gamma}^\alpha \)) with exponent \( \alpha = -0.81 \) (within the range of 1 to 400 s–1). This corresponds to the classical description of xanthan gum solutions.

This finding led many authors to assume the existence of a yield stress that would assume a limiting slope of –1 at a low shear rate. Addressing this issue, however, requires knowledge of the flow curve at very low shear rates which cannot be accessed through classical viscosity measurements. Creep experiments make it possible to perform long-term measurements at a given shear stress and therefore to access to the steady regime (see Section 7.2.2.3.2).
From these measurements, it is possible to explore the low shear rate range. Furthermore, the overall flow curve could be described using the Cross equation [37]. It is worthwhile to note that $m = 0.82$ was very close to $-\alpha$ from the above-mentioned power law equation. The overall shape of this flow curve does not differ basically from that of the other polysaccharides. The main difference lies in the very high apparent viscosity at low shear rate due to the rigidity of the macromolecule.

Another point worthy of interest is related to the variations of $\eta_{sp0}$ as a function of the reduced viscosity, $c[\eta]$, as illustrated in Figure 7.8, where the data obtained for one xanthan sample are compared to the previous master curve for galactomannans (from Figure 7.6). We have the same type of variation, with three concentration regimes in relation to $c[\eta]$ [38]:

\[
\begin{align*}
  &c[\eta] < 1.4 \quad \text{dilute regime (slope = 1.25)} \\
  &1 < c[\eta] < 5.5 \quad \text{intermediate regime (slope = 2.1)} \\
  &c[\eta] > 5 \quad \text{semidilute regime (slope = 4.2)}
\end{align*}
\]

\[\text{FIGURE 7.8 } \eta_{sp0} \text{ as a function of } c[\eta] \text{ for a xanthan gum in solution. Comparison with the master curve obtained with guar gum (straight lines) (see Figure 7.6). (From Cuvelier, G. and Launay, B., Carbohydr. Polym., 6, 321–333, 1986. With permission.)}\]
Here, again, xanthan gum does not differ basically from the polysaccharides in coil conformation despite the peculiar conformation of the macromolecule; however, and quite unexpectedly, the slope in the semidilute regime is lower. Due to the relatively high intrinsic viscosity of the usual samples ($\eta \approx 30$ to $60$ dL/g), entanglements and resulting properties will appear at concentrations on the order of 0.017 to 0.033% ($c^* \approx 1/60$ or $1/30$). The molecular meaning of such phenomena is discussed below with regard to viscoelastic behavior.

### 7.2.2.3 Viscoelastic Behavior

#### 7.2.2.3.1 Dynamic Properties

Figure 7.9 illustrates the viscoelastic spectrum of a 2% (w/w) locust bean gum solution. Comparable results can be found in the literature for other polysaccharides in solution, such as guar gum and pectins, among others [18,26], and can be interpreted. At low frequency, the loss modulus $G''$ is higher than the storage modulus, and both parameters vary sharply with frequency: $G''(\omega)$ and $G'(\omega^2)$. The behavior is said to be liquid-like. As frequency increases, $G'(\omega)$ crosses $G''(\omega)$; the response of the material beyond this cross-over frequency is said to be solid-like. Such behavior is typical of macromolecular solutions with topological entanglements. Within the frequency range explored, we observe the terminal zone (at low frequency) and the beginning of the plateau zone (at high frequency) of the complete viscoelastic spectrum. This means the rheology of polysaccharide solutions is mainly governed by the degree of entanglement of individual macromolecules. The same figure illustrates the
application of the Cox–Merz rule in the case of macromolecular solutions by comparing the $|\eta^*(\omega)|$ curve with $\eta(\dot{\gamma})$, where $|\eta^*(\omega)|$ is the modulus of the dynamic viscosity; $\omega$, the angular frequency; $\eta$, the apparent viscosity; and $\dot{\gamma}$, the shear rate. According to the Cox–Merz rule, the $|\eta^*(\omega)|$ vs. $\omega$ curve should be superimposed on the flow curve over the entire frequency (and shear rate) range. The superposition is actually experienced only in the Newtonian zone and at the beginning of the non-Newtonian one. Exopolysaccharides have been shown to exhibit quite similar viscoelastic properties, as could be expected [34–36]. It is clear that all these polysaccharide are rather flexible and adopt a random coil conformation in aqueous solution.

The viscoelastic properties of xanthan gum again differ significantly from this typical behavior if the concentration is high enough. This is illustrated in Figure 7.10 for a 0.5% xanthan solution. The difference in viscoelastic properties with respect to the other nongelling polysaccharides is clearly seen, as xanthan shows little frequency dependence on $G'$ and $G''$. Moreover, $G'$ is higher than $G''$ over most of the frequency range investigated (10$^{-3}$ to 100 rad s$^{-1}$). Such behavior may appear to correspond to a weak gel [39,40]. It has been suggested that these peculiar properties are due to associations of ordered chain segments giving rise to a weak three-dimensional network [27,41]; however, another interpretation can be proposed [42]. This mechanical spectrum can be regarded as that of a macromolecular solution with the cross-point of $G'$ and $G''$ occurring at a lower frequency than is accessible; therefore, the frequency range accessed allows measurements to be performed in only the plateau zone. The peculiar viscoelastic properties of xanthan can be related

![Figure 7.10](image-url)
only to the relatively high stiffness of the polysaccharide, thus the main
difference in viscoelastic behavior between polysaccharides in coil confor-
ma
tion, such as galactomannans, and xanthan lies in the fact that xanthan chains
adopt a helical conformation that is much stiffer. This rigidity implies a much
more limited mobility of the chains and hence much longer relaxation times.
This difference can be roughly estimated from the cross-point of the \( G' - G'' \)
curves as well as by the departure from the Newtonian zone in the viscosity
measurements (see above); therefore, the so-called “weak gel” properties of
xanthan solutions are only apparent from viscosity measurements, as is the
“yield stress.”

These systems do not display solid-like properties, as is often claimed. A
possible explanation for such peculiar viscoelastic behavior is that xanthan
molecules exhibit the behavior of liquid crystals, as they consist of a nematic
liquid crystalline phase dispersed in an isotropic phase \[43,44\]. Support of
this theory can be found by considering the behavior of \( \kappa \)-carrageenan in the
presence of NaI. Under these specific conditions, it is known that iodide ions
bind to and stabilize \( \kappa \)-carrageenan helices and prevent the aggregation of
helices that is needed for \( \kappa \)-carrageenan helices to yield a three-dimensional
structure. The viscoelastic properties of these dispersions has been shown to
be similar to that of xanthan \[45,46\]; therefore, it can be postulated that these
viscoelastic properties originate from a similar mechanism, which is related
to the high stiffness of the molecules. Furthermore, it has been shown that
low-molecular-weight \( \kappa \)-carrageenan in NaI is prone to form nematic liquid
crystalline mesophases \[47,48\], as does low-molecular-weight xanthan \[44\].
These similarities give support to the above-mentioned interpretation, as nei-
ther xanthan dispersions in salted water nor \( \kappa \)-carrageenan in NaI exhibit weak-
gel properties. They should be classified as concentrated macromolecular
solutions of highly rigid macromolecules \[46\].

7.2.2.3.2 Transient Behavior

The viscoelastic behavior of macromolecular systems can be usefully charac-
terized by means of transient methods such as stress relaxation measurements
or creep-recovery tests. Quite a few examples in the literature deal with this
type of measurements for solutions. Figure 7.11 illustrates the creep-recovery
behavior of locust bean gum at a 2% concentration (same sample as in Figure
7.9). The behavior was typical of a viscoelastic liquid with limited elasticity.
The creep curve, resulting from the application of the shear stress, appears
almost linear except for very short periods when a slight curvature appears
(which is not readily apparent in the figure due to the scale). The linear part
of the curve corresponds to the steady flow regime, and from the slope of the
straight line we can estimate the corresponding shear rate. The recovery curve,
the shear stress being cancelled, showed slight elasticity for a very short time
and then a plateau related to the steady flow previously experienced. The
behavior of a 0.5% xanthan gum is shown in Figure 7.12. The elasticity is
more pronounced and could be classified into instantaneous elasticity and retarded elasticity. The steady flow appeared predominant at times longer than 100 s. The recovery curve again confirmed the elastic character of the system, but the fact that the compliance did not return to zero is consistent with the fact that flow has occurred. Such behavior is typical of a viscoelastic liquid, which exhibits no solid-like character. It is important to note that the shear rate was of the order of $10^{-3}$ s$^{-1}$ in this example. In other words, in the case of xanthan, steady flow can take place over a long period of time.

Again, $\kappa$-carrageenan in the presence of NaI displayed a similar behavior; it did not exhibit any evidence of a solid-like behavior at long timescales [45].
This confirms discussion in the previous paragraphs regarding ruling out the solid-like character of xanthan in aqueous medium and the absence of a true yield stress. From such a determination at different shear stresses ($\sigma$), it is possible to determine $\dot{\gamma}$ and hence $\eta_0 = \sigma/\dot{\gamma}$ in order to investigate the flow behavior at a very low shear rate; therefore, measurements at higher shear rates from classical viscometry can be usefully complemented, as illustrated in Figure 7.7 for a 0.8% xanthan solution [37].

7.3 GELLING SYSTEMS

7.3.1 GENERAL CONSIDERATIONS

7.3.1.1 Weak Gels and Strong Gels

Gels are classically defined as a range of substances that exhibit solid-like properties even though a vast excess of solvent is present. Gelation of polysaccharides arises from physical cross-linking through polymer–polymer interactions. These systems are classified as physical gels. Typically, polysaccharide gels contain more than 90% by weight of water or aqueous electrolyte. Two classes of networks are often distinguished, referred to as “true gels” and “weak gels,” depending on their macroscopic behavior. As a general rule, a true gel should be freestanding and arise from a three-dimensional network. In contrast, the solid nature of a weak gel is less apparent, as it will exhibit flow when submitted to high enough stress. A tenuous, gel-like network may thus exist, but it is easily broken. Aqueous dispersions of xanthan gum are often described as weak gels; however, as we discussed earlier (see Section 7.2.2.3.2), such systems do exhibit all the features of a macromolecular solution. They do not display any solid-like behavior and should not be considered as weak gels. Examples of weak gels are given below.

A classification of biopolymer gels was proposed by Clark and Ross-Murphy [49], who made a distinction between networks formed by gelation of disordered polymers (e.g., gelatin, carrageenans, alginates, pectins, amylose, starch) and systems that involve specific interactions between denser and less flexible particles (e.g., thermally denatured globular proteins, aggregated proteins from enzymic or chemical action). In the first category, the transition is induced by decreasing the temperature (gelatin, carrageenan) or by changing the solvent composition and introducing specific ions (alginites, low-methoxyl pectins). These gels can be considered to be homogeneous at the molecular level and have been described as “association networks” [50]. In the second category, gels are described as particulate, gelation being induced by increasing the temperature (denaturated protein gels) or by enzymatic means (rennet milk setting) [40,51]. Of course, polysaccharide gels all belong to the first category. In all cases, the cross-links between chains originate from physical interactions,
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often hydrogen bonds, but electrostatic, Van der Waals, and hydrophobic interaction forces may also be important.

In the case of alginate and low-methoxyl (LM) pectin gels, for example, the interactions are of the ion bridging type, involving the presence of specific divalent cations, such as Ca$^{2+}$. The energy of these forces is very low compared to covalent bonds and, in order to have stable cross-links between chains or at least cross-links having characteristic lifetimes much longer than the observation time, it is necessary to have a sufficient number of adjoining bonding spots between two chains or more. Thus, the cross-links are not limited to points on the chains but correspond to more or less extended junction zones. In actuality, the cross-links are not permanent and are likely to break and reform continuously. Such systems behave as solids, provided the timescale of the observation is shorter than the lifetime of the physical cross-links. Typically, a physical network would behave as a solid for short periods of observation (or higher frequencies), whereas it may appear as a viscoelastic fluid over longer periods of time (or lower frequencies).

7.3.1.2 Practical Aspects of Rheological Characterization

Viscoelastic measurements of biopolymer gels have been discussed several times in the literature [49,52–56]. A complete characterization would ideally imply that measurements have been performed over several (6 to 10) decades of time or frequencies in order to describe the entire viscoelastic spectrum. Such a characterization may be achieved by using a combination of different techniques [53]; however, classic dynamic measurements are able to examine frequencies from approximately 0.01 to 100 rad/s, a range that corresponds to a timescale range of about $10^{-2}$ to 100 s. Transient methods (creep and stress relaxation) provide information for timescales ranging from 10 to ~$10^5$ s. Access to much shorter timescales (or higher frequencies) can be achieved using a piezorheometer (up to $10^5$ rad/s) [57]. Intermolecular interactions are observed as cross-links when the timescale of experiments is shorter than the relaxation time of these interactions. For these reasons, investigations on gels should be performed using transient methods in order to study the slow molecular motions likely to take place in the gel network rather than by means of dynamic measurements; however, dynamic methods are far more popular because they are more easily carried out.

Among the problems encountered in the characterization of polysaccharide gels, the preparation and molding procedures must be carefully controlled and standardized, particularly with regard to the thermal history. In addition, water losses during measurements have to be avoided. Water loss can be prevented by covering the surface of the sample with a light oil (e.g., liquid paraffin). Another difficulty is related to the syneresis observed for some of these
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systems. This phenomenon results in the exudation of water at the surface of the gel, giving rise to a film that prevents adhesion between the gel and the surface of the measuring device. Slippage can thus be encountered and yield erroneous measurements. This problem can be overcome by roughening the surface of the measuring attachment.

Many different instruments are now available in oscillatory shear to characterize the viscoelastic properties of food gels. Sometimes, when one is dealing with strong gels displaying freestanding properties, simple compression can be used and some experimental devices are available. Indeed, viscoelastic measurements must always be performed in small deformations so the conditions of linear viscoelasticity are fulfilled.

In most cases, the studied sample is poured in a liquid state into the gap of the measuring cell (coaxial cylinders, cone-plate, or parallel plates) where gelation takes place. Gel formation may be triggered by a decrease in temperature (e.g., κ- or ι-carrageenans, biopolymer mixtures) or by the slow release of Ca++ ions (e.g., alginate, LM pectin). It may be necessary to scan the gel for many hours before it reaches a true or a pseudoequilibrium state; therefore, the sample must be protected against dehydration by coating the free surface of the sample.

### 7.3.2 Rheological Characterization

#### 7.3.2.1 Gel Cure

A very popular way to evaluate the gel cure of biopolymers is to measure the dynamic mechanical properties, either $|G^*|$ or, most often, $G'$ as a function of time. One of the main advantages of this approach lies in the fact that, contrary to viscosity determinations, it is possible to characterize the evolving system without disturbing it due to the small deformation amplitudes used. The non-disturbing character of this method cannot be assumed in every case, and it is advisable to verify it whenever possible. Another point to check is that the linear viscoelastic range has not been exceeded, because outside of this range the results will also depend on the deformation amplitude in a manner likely to change from the beginning to the end of the curing process. As the linear viscoelastic range may decrease during gelation, it is not always easy to ascertain that this essential condition is met.

The gel cure of many polysaccharides is followed by $G'(t)$ measurements of the gel formed by cooling, as in ι-carrageenan [58] or κ-carrageenan [59], or by the slow release of Ca++ ions, as in LM pectin or alginate gels [60,61]. An example is given in Figure 7.13 for alginate gelation. A progressive cross-linking mechanism takes place. Larger and larger clusters of associated or aggregated chains are formed until reaching a critical point, the gel point, where the largest cluster spans. At this point, the viscosity tends toward an infinite value, and $G_o$, the equilibrium shear modulus, diverges from zero [62]. At the gel point, the rheology of the system changes from that of a viscoelastic...
fluid to a viscoelastic solid. At the critical gel point, the system is wall-to-wall connected (percolation threshold) and is characterized by a critical behavior with $G'(\omega)$ and $G''(\omega)$ obeying the same power law:

$$G'(\omega) \sim G''(\omega) \sim \omega^n$$

$$\tan \delta_c = \frac{G''(\omega)}{G'(\omega)} = \text{constant} = \tan \left( \frac{n\pi}{2} \right)$$

Such changes allow determination of the critical gelling time from measurements of $G'$ and $G''$ as a function of frequency at different times. Such a procedure has been described for t-carrageenan [63], and details of the procedure are given in Section 7.3.2.5. A prerequisite of such measurements is sufficient sensitivity of the rheometer in the sol state (below the gel time). Also, the gelling process has to be slow enough with respect to the duration of measurements at the lowest frequency. Very often, this is not the case, and, for these practical reasons, most authors define the gel point as the time where $G' = G''$. Beyond the gel point, the incipient network is progressively reinforced by incorporation of increasing amounts of material into the gel phase, stiffening the elastic properties of the gel. A quasi-stable rheological state is reached after a sufficient amount of time has elapsed and if there are no disturbing phenomena, such as syneresis. By analogy with a polymerization mechanism, such a gelation process can be viewed as resulting from an increase in network connectivity.
7.3.2.2 Mechanical Spectra

At the end of the gel cure experiment, a more complete characterization can be achieved by way of the frequency dependence of $G'$ and $G''$. A typical example of mechanical spectra is shown in Figure 7.14 for a κ-carrageenan gel. Many other examples for polysaccharide gels in oscillatory shear are available in the literature. Agar–agar gels investigated in uniaxial deformation exhibited a similar pattern [65]. It is a general rule that the mechanical spectrum of polysaccharide gels is characterized by a flat dependency of the shear storage modulus ($G'$) over the entire range of accessible frequencies. Very often, the loss modulus variation is not described. Due to its low value with respect to the storage modulus ($G' \gg G''$), $G''$ is considered to be less important than $G'$. Several times, however, a minimum in the loss modulus has been reported at a low frequency. This may have a molecular meaning but has not yet been discussed in detail.

Most of the time, the frequency range available in classical rheometers is limited to three or four decades (at most, between 0.01 and 100 rad/s). The generality of the behavior, typically the flat frequency dependence and $G' > 10G''$, means that dynamic measurements can be utilized to provide an unambiguous, operational definition of true gels as opposed to weak gels and macromolecular solutions [49,66]. As a matter of fact, a highly viscous macromolecular solution, due to a high degree of overlapping of macromolecules, apparently may not exhibit flow and may be quite difficult to differentiate from a gel on the basis of a visual observation only. Dynamic measurements can be very useful in this respect, as illustrated by a comparison of Figure 7.14 with Figure 7.9.
7.3.2.3 Mixed Gels and Composite

The mixing of two polysaccharides in solution may lead to gel formation. Xanthan–locust bean gum and κ-carrageenan–locust bean gum are well-known examples of such gels. The simplest two-component gelled systems should be obtained by mixing two biopolymers, one exhibiting gelling properties and the other not. This is the case of carrageenan–locust bean gum mixtures. Figure 7.15 shows the mechanical spectrum of a κ-carrageenan–locust bean gum (80/20) mixture as compared to κ-carrageenan alone [64]. The well-known synergistic effect is illustrated by the rise of the storage modulus due to the presence of the locust bean gum. It can also be seen that the loss modulus $G''$ and the $G''/G'$ ratio were higher for the mixture than for the carrageenan. This may reflect the fact that nongelling material increases the damping factor of a gel. It can also be observed that the storage modulus increases slightly with frequency whereas the loss modulus shows a maximum at about 1.2 Hz and then decreases. This example clearly illustrates that a mixed gelled system can have viscoelastic properties far removed from those of the only gelling agent of the mixture.

Figure 7.16 shows the results obtained with a xanthan–locust bean gum mixture. The gelling ability of such a system is well known, although xanthan gum, as discussed above, does not really gel by itself nor does locust bean gum. $G'$ remains constant at low frequencies, up to 1 rad/s, and then increases with frequency, with an inflection point at about 4 rad/s; thus, two elastic
plateau zones are observed. On the other hand, $G''$ exhibits a maximum at a frequency that roughly coincides with the inflection point of the $G'$ vs. frequency trace. This maximum in $G''$ can be ascribed to a relaxation process for which the characteristic time can be estimated by the inverse of the frequency. Moreover, at constant temperature, the viscoelastic properties change for a long period of time, with $G'$ increasing at high frequencies but decreasing at low frequencies [32]. Consequently, if $G'(t)$ is used to follow gel cure in such systems, the results will be strongly dependent on the choice of the frequency of measurement. This uncommon behavior is attributed to the coexistence of two types of junction zones, with a slowly changing balance between one type and the other [67]. The low-frequency plateau arises from stable physical cross-links, whereas the second plateau, which appears at high frequencies, can be attributed to cross-links that appear to be less permanent.

In the presence of proteins, phase separation may take place, giving rise to a composite system for which the rheological behavior can be considered to be that of a suspension of particles in a macromolecular solution [68] or a more complex gelled system, particularly in the case of denatured globular proteins [69,70]. Dynamic measurements can be very useful in the description of such systems; however, additional techniques, such as microscopic tools, are required to gain an understanding of the underlying mechanisms [68].

When both components can gel by themselves, the resulting system is usually described as a composite. Agar–gelatin, gelatin–alginate, gelatint–carrageenan, and gelatin–gellan mixtures are examples of such systems that

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**FIGURE 7.16** Mechanical spectrum of a 70/30 xanthan–locust bean gum mixture (0.5% total polysaccharide concentration in 0.1-M NaCl). $G'$, □; $G''$, ■. (From Cuvelier, G., Propriétés fonctionnelles de la gomme xanthane, propriétés rhéologiques en solution aqueuse et interactions avec la gomme de caroube, Ph.D. dissertation, Université Paris XI, France, 1988. With permission.)
can be used by the food industry. These systems are biphasic, with each component gelling in its own phase [71–73]. The composite, then, can be described as a continuous matrix enriched in one of the components filled by gelled droplets enriched in the other. Its behavior will thus be governed by the original composition of the mixture. A variety of properties can be expected, particularly in the vicinity of the composition for phase inversion. A large number of parameters (e.g., pH, ionic strength, composition, temperature) can be involved in determining the final properties and, therefore, the texture of such systems. Manipulating these parameters is likely an interesting way to develop novel textures; however, information related to the dynamic behavior of this type of system is quite poor. It is generally limited to the description of $G'$ variations as a function of composition [72–76]; however, some results have been reported regarding mechanical spectra.

Clark et al. [71] described the dynamic properties of agar–gelatin co-gels (1% agar and 10% gelatin). The mechanical spectrum was close to that of gelatin alone, suggesting that the gelatin molecules could be implied in the continuous matrix. The agar molecules were located in rigid beads that played the role of fillers in reinforcing the continuous network. Horiuchi [76] reported data on a gelatin–agar mixture (15% gelatin and 1.5% agar). No frequency dependence within the frequency range 0.1 to 10 Hz was reported, but a sharp increase in the complex moduli was found between 50 and 100 Hz. Similar patterns have been reported for $\kappa$-carrageenan–locust bean gum mixed gels [77]. The frequency dependence may thus be taken as an indication of the heterogeneity of gelled systems.

### 7.3.2.4 Weak Gels and Fluid Gels

It is more difficult to give a clear definition of these systems than for true gels. Macromolecular solutions can be confused with a weak gel, as we discussed above in the case of xanthan gum; however, systems do exist that have properties between those of true gels and macromolecular solutions. Examples of weak gels with alginate at a low Ca$^{++}$ content, t-carrageenan, or gellan have been described [78–82]. For example, 0.005% concentrated gellan gum in 10-MM CaCl$_2$ displayed a mechanical spectrum typical of a true gel but with $G'$ on the order of 0.2 Pa [82]. These systems are used as shear-reversible gels in dairy products (multilayer desserts) or for the stabilization of particles in an apparently fluid medium [80]. They lose their rigidity upon shearing during the process and then recover part of their gel properties almost as soon as the shearing action is removed. Such gels are often referred to as thixotropic. Some of them are also referred to as fluid gels. The sensitivity of such systems to shearing as well as the timescale of recovery after shearing can be monitored using dynamic viscoelastic measurements [79]. Specific rheological procedures have been proposed to characterize these systems by determining their viscoelastic properties as well as their behavior at large deformations [81,83].
Another kind of weak gel can be obtained in the case of polysaccharide mixtures where one of the polymers predominates. Figure 7.17 illustrates the properties of a mixture of xanthan with locust bean gum at 0.5% total concentration at a xanthan-to-galactomannan ratio of 5/95 [42]. Locust bean gum represents 95% of total polysaccharides of the system. For comparison, the viscoelastic spectrum of the galactomannan at the same total concentration is also shown. A dramatic change in the $G'$ trace is apparent, while the $G''$ curve is only slightly shifted to higher values. The main feature is the flattening of the $G'$ curve toward the low-frequency range, where it plateaus, contrasting strongly with the $G''$ variations for galactomannan. This means that the behavior is solid-like and can be confirmed unambiguously from creep-recovery experiments [42,84]. Similar results have been obtained in the case of xanthan–guar gum mixtures, suggesting that the underlying mechanism is not governed by the chemical structure of the galactomannan (i.e., the mannose-to-galactose ratio) [84].

Similar effects can be observed when galactomannans are mixed with polysaccharides other than xanthan. Figure 7.18 shows a κ-carrageenan–locust bean gum mixture in 0.13-Μ KCl [85]. The κ-carrageenan-to-galactomannan ratio is as low as 1/99. It can clearly be seen that the presence of a small amount of the carrageenan strongly modifies the properties of the locust bean gum. Here, again, despite the low amount of κ-carrageenan in the mixture, gel-like properties are exhibited; however, the viscoelastic properties of such systems are far removed from those of true gels. Instead, they exhibit properties...
close to the galactomannan solution at high frequencies (long timescales) and to a gel at low frequencies (short timescales). Furthermore, at low frequencies, the storage modulus $G'$ related to the density of the network remains very low. In other words, the network is tenuous and is spread all over the macromolecular solution of the major polysaccharide.

### 7.3.2.5 Effect of Temperature

Many polysaccharide gels are thermoreversible. Their melting (or gelling) can be monitored by means of dynamic measurements that follow sol–gel transitions. In most cases, researchers perform measurements as a function of temperature at a given frequency. They define the cross-point $G' = G''$ as the critical point. Similar to the case for gel cure (see Section 7.3.2.1), a critical behavior exists at the percolation threshold that allows one to determine the critical melting, or gelling, temperature of a polysaccharide gel. An example is given in Figure 7.19 and Figure 7.20 for a thermoreversible $\kappa$-carrageenan gel. Measurements have been performed during the gelling process, with the temperature being decreased step by step. An equilibrium mechanical spectrum was obtained at every temperature. Figure 7.19 shows three mechanical spectra close to the sol–gel transition. At 55.3°C, the system exhibited the viscoelastic properties of a solution, while at 51.5°C weak gel properties were clearly apparent. At the gel point (53.5°C), a power law was obeyed for $G'(\omega)$ and $G''(\omega)$ with exponent $n$ at 0.42. Because at this stage the critical phase angle $\delta_c$ (tg $\delta = G''/G'$) is independent of frequency, a precise determination of the
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7.3.2.6 Rheology of High Sugar/Polysaccharide Systems

Dynamic oscillation can be employed to investigate changes in the mechanical properties of hydrocolloids in the presence of sugars. The effect of sugars at concentrations lower than 40 to 50% is generally moderate. The only reported changes in the case of gelling polysaccharides (gellan, carrageenans) are an increase of the gelling temperature and of the $G'$ modulus without any dramatic change in the shape of mechanical spectra [89]. In the case of HM pectins in an acidic medium, similar trends were observed at concentrations between 50 and 74% [90]. With an increase in sugar content, up to 85% in certain studies (often achieved by using a mixture of sucrose and glucose syrup), dramatic changes have been reported, whatever the type of polysaccharide.

When dealing with gelling polysaccharides, the mechanical spectrum is dramatically changed, as the system undergoes a transformation from a rubber-like consistency to a glassy state. The mechanical properties deviate from those of a rubbery structure at high temperatures and, upon cooling, the system transforms to a glassy consistency. It has been demonstrated that the formation of a cohesive polysaccharide network seems to be a prerequisite for the

FIGURE 7.19 Effect of temperature on the viscoelastic properties of an ι-carrageenan gel (0.8% in aqueous 0.2-M NaCl) close to the gel point (upon cooling). $G'$, open symbols; $G''$, closed symbols. Squares, 55.3°C (above the gel point); triangles, 53.5°C (at the gel point); circles, 51.7°C (below the gel point). (From Michon, C. et al., in Food Macromolecules and Colloids, Dickinson, E., Ed., Royal Society of Chemistry, Cambridge, 1995, p. 462. With permission.)

10⁻³ 10⁻² 10⁻¹ 10⁰ 10¹ 10²
$G'\, G''$ (Pa)

Frequency (rad/s)

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acceleration of vitrification phenomena in high-sugar–polysaccharide systems [91]. Applying the so-called time–temperature superposition procedure to synthetic polymers made it possible to build master curves over more than 9 decades of frequencies covering the end of the plateau modulus, the rubbery zone, and the transition toward the glassy zone. These effects have been analyzed in detail within the framework of current theories applied to high-molecular-weight synthetic polymers [92,93]. Overall, it is clear that, at a low sugar content (less than 40 to 50%), the behavior is reminiscent of that of the polysaccharide in water, but the behavior of high-sugar–polysaccharide systems cannot be predicted on this basis. Recently, the generality of these phenomena has been challenged in a study on gellan–sugar systems [94]. It was hypothesized that under certain conditions gellan forms “gel particulates” embedded within the cosolute matrix rather than a continuous network.

7.4 CONCLUSION

Rheological methods are very powerful techniques that provide a complete description of the thickening and gelling properties of polysaccharides. Polysaccharides can result in three types of systems (in increasing order of the structure of the medium): macromolecular solutions, weak gels, and strong gels. Distinguishing among these three types of systems can be easily accomplished using viscoelastic measurements. Dynamic methods are more generally used because

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they are more easily performed. Transient methods such as creep-recovery or stress relaxation are employed less frequently because they require longer experiments; however, they can be helpful when some ambiguity remains regarding the structure of the medium. Employing rheoptical methods and microrheology for the study of biological soft materials should also be useful in furthering our understanding of these systems [95–97].

It is obvious that the rheological properties are directly related to the molecular interactions occurring in the system. When dealing with macromolecular solutions, the mobility of the chains is dependent on the degree of entanglement of the chains, thus flow and viscoelastic properties that are typical of such systems can be determined and cannot be confused with those of other systems. Galactomannans (locust bean gum, guar gum), λ-carrageenan, and cellulose derivatives (carboxymethylcellulose, hydroxyethylcellulose) yield such properties in an aqueous medium. Despite its specificity, xanthan gum also belongs to this category. In fact, the low mobility of xanthan chains due to their semiflexible nature is enough to explain the specific properties of such macromolecular solutions.

In the case of gels, the three-dimensional network is structured by junction zones arising from specific interactions between polymer chains. These are low-energy interactions, and a cooperative effect has to be involved in order to explain the absence of mobility of these chains. The structure of the network can give rise to several types of interactions with different lifetimes. For true gels, the fact that $G'$ is independent of frequency and much higher than $G''$ means that interactions responsible for the observed elastic properties have long enough lifetimes when compared to the timescale of measurements ($1/\omega$); in other words, no relaxation process takes place within this period. It may be expected that junction zones are permanent (infinite lifetime); however, any relaxation process with a lifetime longer than $\sim 10^3$ s is not accounted for in classic dynamic measurements. It is thus clear that ambiguity always remains with regard to the existence of a permanent network in such systems.

The situation is far more complex when dealing with multicomponent macromolecular systems involving various types of interactions. Again, viscoelastic measurements are expected to give access to different timescales, providing information on the macroscopic structure of the system. The fact that the dynamic properties of two-component systems differ from those of one-component gels suggests that interactions in mixed systems are numerous and relaxed at rather different times.

Finally, it should be reiterated that a complete description of the functional properties of polysaccharides in a given food system must also take into account other mechanisms, such as, for example, phase separation, depletion–flocculation in dispersed systems, and interactions with milk proteins (casein, β-lactoglobulin) in dairy products, among others.
REFERENCES


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Nondigestible Carbohydrates: Nutritional Aspects

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8.1 INTRODUCTION

Carbohydrates in plant foods can be classified as digestible (monosaccharides, α-linked di- and oligosaccharides, and starch) or nondigestible (dietary fiber, nonstarch polysaccharides, β-linked di- and oligosaccharides, and resistant starch). The major distinction is that the former are hydrolyzed by human digestive enzymes and absorbed in the upper gastrointestinal tract, whereas the latter are not. Digestible carbohydrate is the most important biological fuel provider in human nutrition. Nondigestible carbohydrates can, however, provide some energy (approximately 2 kcal/g in the form of short-chain fatty acids) if they are fermented by bacteria in the colon [1].

Nondigestible carbohydrates could all be categorized as dietary fiber. After considerable deliberation, the American Association of Cereal Chemists (AACC) proposed the following new definition [2]:

Dietary fiber is the edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine. Dietary fiber includes polysaccharides, oligosaccharides, lignin, and associated plant substances. Dietary fiber promotes beneficial physiological effects such as laxation and/or blood cholesterol attenuation and/or blood glucose attenuation.

Our concept of dietary fiber is thus changing. Although the majority of nondigestible carbohydrate (nonstarch polysaccharides and resistant starch) in our diet is obtained from the consumption of plant foods, isolated nonstarch polysaccharides (hydrocolloids of vegetable, animal, microbial, or synthetic origin) are used in small amounts to control the functional properties of aqueous foodstuffs. They may also be consumed in larger quantities for their therapeutic properties. Additionally, nondigestible oligosaccharides such as galacto- and fructooligosaccharides are described as having prebiotic properties; that is, they promote the growth of lactic acid bacteria (probiotics) in the gut [3,4]. These prebiotics are increasingly being employed in the functional food market.

The first source of dietary nondigestible oligosaccharides is mother’s milk, which contains up to 100 different types [5]. They also occur naturally in many common foods, including vegetables, fruits, and honey [6], and can be manufactured from simple sugars by transglycosylation using synthesizing enzymes [7,8] or by controlled degradation of various nonstarch polysaccharides using either acid or enzymatic hydrolysis [6,9]. Although oligosaccharides are generally chemically defined as glycosides that contain between three and ten sugar units, some nondigestible (β-linked) disaccharides such as lactulose are often classified commercially as oligosaccharides because they have functionality similar to that of the longer chain nondigestible oligosaccharides [10].

Adequate consumption of nondigestible carbohydrates is considered to confer many health benefits, and epidemiological studies suggest an inverse relationship between a higher intake of dietary fiber and the risk of diseases.
such as colorectal cancer [11] and coronary heart disease [12]. Nondigestible carbohydrates may affect digestion and absorption in the upper and lower gastrointestinal tract, postprandial plasma levels of glucose, insulin, lipids and cholesterol, satiety, and energy balance, as well as stool output, colonic microflora, and their fermentation products. The physicochemical properties of a polysaccharide govern its physiological effects, but it is difficult to predict the specific actions of a carbohydrate without knowledge of how it interacts with other food components, digestive secretions, and the colonic bacterial flora. Furthermore, the functionality of a nondigestible carbohydrate that forms an integral part of a food matrix may differ significantly from that of isolated gums and is dependent on physical and chemical interactions within the food as it passes down the gut.

8.2 ACTIONS IN THE GASTROINTESTINAL TRACT

Nondigestible carbohydrates have great potential for modulating the digestion, absorption, and postprandial effects of food. The rate and extent of absorption and the metabolic impact of a nutrient will depend on the site in the gut from which it is absorbed, the physical intactness of the food, and the physicochemical properties of the individual nondigestible carbohydrates with which it is ingested. The possible actions of undigested carbohydrates on the gastrointestinal tract are listed in Table 8.1.

8.2.1 FOOD PROCESSING AND COOKING

The first influence on the action of nondigestible carbohydrates in the gut occurs during food processing and cooking. Particle size, porosity, and cohesion play a role in controlling their transit through the gastrointestinal tract, enzyme accessibility, fermentation, and fecal excretion [13]. Processing can reduce particle size, increase porosity, and solubilize components within the cell wall involved in the cohesiveness of its matrix. Plant cell walls that are intact can act as physical barriers that protect starch and other nutrients from digestive enzymes. The robust cell walls of unprocessed foods have greater resistance to chewing and gastric disruption than plant cell walls that have been softened or disrupted by cooking or grinding. This resistance reduces the release of nutrients such as beta-carotene [14], as well as the digestibility of starch.

The physiological actions and fate of plant cell-wall material or associated polysaccharides are determined in part by their hydration properties. Consumption of insoluble plant polysaccharides, which do not form part of an intact cell-wall structure, has little effect on digestion. In contrast, isolated soluble polysaccharides can greatly reduce the rate of small intestinal digestion and absorption [15–17], especially if they generate a high viscosity; however, some of this effect may be due to integration of the soluble fiber within the food matrix. Brennan et al. [18] studied the effect of guar galactomannan on
wheat bread digestibility and reported significant attenuation of starch hydrolysis in vitro and a reduction in postprandial glucose levels in the pig. Microscopic analysis of the guar bread revealed that the starch granules were glued together or coated with galactomannan, which may act as a physical barrier to prevent α-amylase–starch interactions or the release of hydrolysis products. The effect on postprandial glucose of viscous polysaccharides given as a supplement is small unless the polysaccharide is mixed with the food [19]. Moreover, similar polysaccharides contained in natural foods (i.e., bound within the plant cell wall) may have little effect on digestibility if they are not available for association with water and other nutrients.

TABLE 8.1
Possible Actions of Nondigestible Carbohydrate in the Gastrointestinal Tract

Effects on Transit Time
Reduces gastric emptying (soluble fiber)
Stimulates colonic propulsion (insoluble fiber)
Modulates small intestine transit time
Increases stool output and frequency
Reduces whole gut transit time

Effects on Digestion
Reduces chewing efficiency
Impairs gastric disruption
Reduces luminal mixing (viscous fiber)
Reduces interaction between enzymes and substrates (viscous fiber)
Reduces interaction between receptor and stimulus (viscous fiber)

Effects on Absorption
Interacts with mucus
Inhibits flow
Apparently increases unstirred layer (viscous fiber)
Increases rate of glucose absorption
Decreases fat absorption
Increases delivery of nutrients to distal ileum (viscous fiber)
Increases delivery of substrates to colon

Colonic Effects
Dilutes colonic contents
Stimulates bacteria activity
Produces short-chain fatty acids
Promotes growth of specific bacterial species
Acts as a prebiotic (oligosaccharide)
Reduces colonic pH
Increases colonic absorption of minerals
Increases mucosal growth and thickness
Isolated nondigestible carbohydrates are increasingly being incorporated into foods as the industry strives to obtain new or modified textures. This practice can also affect nutrient availability during storage. A classic example of this is the influence of hydrocolloids on recrystallization or retrogradation of starch (formation of type III resistant starch). Some hydrocolloids, such as konjac glucomannan [20] and xanthan [21], have been reported to inhibit starch recrystallization during long-term storage, whereas others, such as guar and carrageenan [21], reportedly hasten retrogradation. The extent of preingestion processing of the food is, therefore, critical to its ultimate action in the gut.

8.2.2 Effects on Chewing and Palatability

Chewing disrupts the food matrix. This releases the flavors and increases texture perception, which will determine the palatability of the food, extent of mastication, and speed of gastric filling. The ease of disruption of plant food is related to its structural integrity. The extent of chewing can determine the rate of digestion and, thus, postprandial glycemia [22]. In contrast, isolated soluble polysaccharides ingested in high quantities are often unpalatable, giving a viscous mouthfeel and aftertaste. This effect can be reduced by their incorporation into foods such as bread [23]. The same polysaccharides used as stabilizers and emulsifiers in foods are generally present in small quantities and have little effect on palatability.

8.2.3 Gastric Emptying

In the stomach, food is subjected to the action of gastric acid and to the vigorous grinding action of gastric motility. The pylorus generally allows food of only less than 2-mm diameter to exit [24]. The bulk of food is stored in the body or fundus of the stomach; when the fundus contracts, it forces food into the much smaller antral/pyloric region. If the pylorus is open and the food is small enough, it will be squirted into the duodenum. If the pylorus is closed or the food particles are too large they are forced back up into the body of the stomach. This constant and powerful movement disrupts the food particles until they are small enough to exit the stomach. The pattern of gastric emptying depends on the liquid/solid nature of the meal. Most of the liquid must be emptied from the stomach before much solid emptying can take place [25]. It is much easier to grind the solid if most of the liquid is emptied out. The action of the plant cell-wall polysaccharide in the stomach is to promote the integrity of the food and to slow disruption. Lignification of the plant cell wall increases its strength and resistance to damage. Isolated soluble polysaccharides, on the other hand, alter the characteristics of the liquid phase and can have major effects on the disruption and gastric emptying of different foods. Most soluble nonstarch polysaccharides form viscous solutions at low concentrations (0.5 to 1%); however, some, such as gum arabic, require much higher concentrations (~12%) to attain a similar viscosity. As the amount of such hydrocolloids added to
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foods is usually low, they normally have little effect in the upper gut. If, however, significant amounts of viscous polysaccharides are ingested (for example, as therapeutic agents), they may slow the gastric emptying of liquids [16]. In contrast, emptying of solids may be accelerated due to lubrication [24]. Not all viscous polysaccharides have these effects, however [26].

8.2.4 SMALL IntESTINE DIGESTION AND ABSORPTION

Digestion in the small intestine progresses as the food interacts with enzymes secreted from the pancreas. The products of digestion must then move from the central lumen, across the mucus layer, to the brush border of the mucosal epithelia, where they may require further digestion by brush-border enzymes. Absorption then occurs by passive, carrier-facilitated, or active transport. Physical or chemical barriers at any of these stages will reduce the rate of digestion and absorption, which will lower postprandial plasma levels of glucose and other nutrients and may have several nutritional sequelae.

The release of nutrients from food ingested as intact plant cells is related to the resistance of the cells to disruption. The nutrients are physically entrapped within the cellular structure and cannot be digested until the cell wall has been breached [14,27]. In the case of starch, the starch granule must also be disrupted. Raw starches, as found in raw potatoes and bananas, are resistant to amylase in the small intestine and enter the colon, where they are fermented [27]. The disruptibility of the plant cell is determined by the cell-wall structure, lignification, particle size [17], cooking, processing [28], and mastication [22].

The glycemic index (a standardized measure of the increase in plasma glucose) of foods varies considerably [29]. Part of this variation is attributed to differences in the disruptibility of the cell wall, although other factors such as lipid content may also play a role. Pulses, such as beans or lentils, have the most resistant cell walls and the lowest glycemic index [29]. Thus, intactness of the cell wall may represent the major effect of a high-fiber diet on absorption of nutrients and should be studied in more detail. The concept of utilizing whole grains — bran, cell walls, starch, phytochemicals, and antioxidants — has become increasingly important when considering the preventative action of high fiber on chronic diseases such as heart disease and cancer. The epidemiological evidence at present supports the suggestion that the protective effects of whole grains are superior to those of dietary fiber [30,31].

Slowing of digestion and absorption in the small intestine by viscous polysaccharides may be due to a combination of the physical entrapment of nutrients, resistance to the mixing movements of intestinal contractions [32], inhibition of enzyme activity [17], and an increase in mucus production [33]. Enzyme activity may be reduced by viscous polysaccharides [17,34], but the pancreas compensates by secreting more enzymes [35]. The major mechanism is likely to be the resistance to the mixing action of intestinal contractions [32], which is related to an apparent increase in the unstirred water layer.
through which molecules can pass only by diffusion [36,37]. As viscosity is largely dependent on the number and size of the polymer chains, these are likely to be critical determinants of the physiological activity of a particular polysaccharide [38]. Although it is believed that soluble fibers increase luminal viscosity, attempts to measure increased viscosity in the gut have not always met with success [39]. Increased viscosities have been reported in the rat gut after ingestion of guar gum, oat bran, xanthan gum, and methylcellulose [40–42]. Ellis et al. [38] reported an inverse relationship between the rate of glucose absorption and viscosity of jejunal digesta in pigs fed guar gum. Cameron-Smith et al. [42], however, found no relationship between intestinal viscosity and glycemic response. This suggests that the immobilization of water in the gut by polysaccharides may be as important as their viscosity; however, partially hydrolyzed guar gum with reduced viscosity, and probably reduced water-sequestering ability, was less efficient in promoting a hypocholesterolemic response in animals than a high-viscosity guar gum [43,44]. This has been attributed to decreased stimulation of hydroxymethylglutaryl coenzyme A reductase (the rate-limiting enzyme in cholesterol synthesis) and decreased fecal bile acid excretion by the lower viscosity gum compared with the higher viscosity gum [43]. However, Levrat-Verney et al. [45], who also found significant reduction in plasma cholesterol in rat, reported no effect on hydroxy-methylglutaryl coenzyme A reductase levels after consumption of low levels of viscous hydrocolloids. A recent in vivo study in both rat and hamster [46] suggests that increased intestinal viscosity is the principal characteristic responsible for increasing fecal sterol output and hence decreased plasma cholesterol. Nevertheless, partially hydrolyzed guar gum is still an effective lipid-lowering agent when consumed with high-fat diets [47,48] and may be acting as a physical barrier.

It is difficult to separate the relative roles of the delay in gastric emptying and the inhibition of digestion in the small intestine in reducing postprandial glycemia. Gastric emptying rate was not found to be related to effects on glycemia in humans [16] for guar gum, but other studies in the dog [49] and in humans [50] found that the role of gastric emptying was significant or even dominant. Whichever mechanism is responsible, the action of these polysaccharides is dependent on their concentration at each particular site of the gut. Even at constant molecular weight, viscosity is not a static measurement and is dependent on concentration, ionic environment, and shear rate [26]. Predicting the action of a viscous polysaccharide from its preingestion viscosity can be very misleading. Gastric acid and the ionic strength of intestinal secretions can reduce the viscosity of some polysaccharides and mixtures, and the large volume of secretion in the duodenum will reduce their viscous properties further [26,42]. This loss of viscosity in the upper small intestine is also reflected in their action on transit time. On the whole, viscous polysaccharides
Carbohydrates in Food tend to delay mouth-to-cecum transit [16, 51, 52]; however, the biggest effects are in the stomach and ileum [52, 53] and in colonic filling [54]. There is little effect in the jejunum.

Most polysaccharides are pseudoplastic (shear thinning) [55]; thus, irrespective of other factors, a reduction in viscosity would be expected to occur along the gastrointestinal tract due to intestinal agitation. Furthermore, the use of instrumental viscosity measurement at given experimental shear rates does not accurately reflect viscosity within the gut because it is impossible to know how intestinal agitation affects shear thinning of the ingested polysaccharide at each site of the gut. Thus, even experimental measurement of viscosity over a range of shear rates cannot truly predict *in vivo* behavior. It is possible to characterize a polysaccharide by its zero shear viscosity [56], which may give some idea of how it will act in the gut, but more studies are needed to establish this. Indeed, in more relevant soluble-fiber-containing foods, interaction with the food matrix may be more important than viscosity [18].

### 8.2.5 Effects on Blood Glucose

The result of a decreased rate of digestion and absorption of carbohydrate in the small intestine is reduced postprandial glycemia and insulinemia. This reduction is of therapeutic use in diabetes [57] but may also provide useful long-term protection from high blood glucose and inappropriate glycosylation of a variety of other molecules, such as collagen [58]. The glycemic index is a useful parameter relating the postprandial blood glucose levels after ingestion of a carbohydrate food to the postprandial glucose seen after ingestion of an equivalent amount of glucose or white bread [29]. This index has been used successfully to predict the effect of mixed meals as well as foods [59, 60], but it will be affected by fat content as well as dietary fiber or type of carbohydrate ingested. The major determinants of the glycemic index of starchy foods are the characteristics of the starch rather than the protein, fat, total dietary fiber, or phytate content [61–63]. The glycemic index does not relate directly to normal food portions, especially for foods containing low amounts of digestible carbohydrate. It is based on the amount of food containing 50 g carbohydrate, and this may be a large volume for some foods. Recently, the more relevant concept of glycemic load has been introduced which multiplies the glycemic index by the amount of carbohydrate in the food. International tables of glycemic load values are now available [64].

### 8.2.6 Effects on Blood Lipids

The effects of nondigestible carbohydrates on fat digestion are similar to those on the digestion of starch; however, the digestion of fat is a more complex process that involves not only pancreatic enzymes but also bile acids and micelle formation. Fat digestion and absorption take place further along the
small intestine and are more likely to be disrupted. Indeed, in most ileostomy studies, viscous polysaccharides are more likely to increase the ileal output of fat than carbohydrate [39,65]. In addition to the action of viscous polysaccharides on motility and mixing, they can entrap bile acids [66–69], which increases fecal bile acid loss and reduces fat absorption by inhibiting micelle formation. Further effects of nondigestible carbohydrates on blood lipids may relate to the short-chain fatty acids (SCFAs) produced during their fermentation, and these are discussed below.

### 8.2.7 Effects on Minerals and Vitamins

Some plant cell-wall polysaccharides, such as pectin and alginates, that contain uronic and phenolic acid groups are able to bind minerals and vitamins and reduce their absorption in the small intestine [70–73]; however, it is generally agreed that in normal adults they have little effect on mineral balance [73] or vitamin status [74]. This may not be true in young children, the very elderly, and other vulnerable groups [75,76]. The extent to which a particular polysaccharide can bind minerals is correlated to its cation exchange capacity [77], but much of the effect of fiber-rich foods on mineral absorption may be due to the associated phytate rather than carbohydrate [78]. A high-fiber diet is known to inhibit fat absorption; thus, it should theoretically inhibit fat-soluble vitamin absorption, but high fiber consumption does not appear to compromise vitamin status [74]. In contrast, some animal [79–83] and human [84] studies have shown consumption of nondigestible carbohydrate to have positive effects on vitamin or mineral balance.

Classic textbook physiology generally describes vitamin and mineral absorption as an upper gut event and gives little reference to the role of the lower gastrointestinal tract in absorption other than that of water; however, microbial synthesis of vitamins such as thiamine, riboflavin, and vitamins B6 and K in the colon is a well-established phenomenon [85–88], and nondigestible carbohydrate has been shown to improve vitamin status in the rat [81]. This may be the result of carbohydrate entering the colon, which increases the numbers of vitamin-producing bacteria and hence the rate of vitamin synthesis and absorption. Likewise, improved mineral status has been demonstrated for nondigestible carbohydrates. Fructooligosaccharides stimulated magnesium absorption to similar extents in cecal-cannulated rat in vivo when administered orally or by cecal infusion [80]. Coprophagy (ingestion of feces by laboratory rats) did not alter magnesium absorption [80]. Soluble arabinoxylans have also been shown to enhance rat cecal absorption of both calcium and magnesium [82]. This may be the result of the acidification of digesta, which increases absorption by several possible mechanisms, including cecal hypertrophy, cation solubilization, and a specific effect of SCFAs. Short-chain fatty acid absorption at acidic pH, in particular, would supply more protons to the exchangers and result in a higher transport rate [82]. A recent investigation of the effects of
fiber and modified starch supplementation of infant formula [89] showed increased calcium availability in vitro when compared to standard formula in the presence of inulin but reduced availability when pectin and locust bean gum were added. Iron availability was reduced in the presence of pectin and oligofructose but increased when pregelatinized starch was the added carbohydrate. Thus, mineral availability can be affected both negatively and positively by the presence of carbohydrate, and the effect is dependent on the nature of both the carbohydrate and the cation [89]. Further research is required as, with the exception of vitamin K, sound evidence is lacking in support of increased colonic absorption of vitamins and minerals being the main mechanism for improved nutrient status in both animals and humans.

### 8.2.8 Colonic Fermentation

Any carbohydrate escaping digestion in the small intestine is subject to fermentation by the colonic bacteria. The products of this fermentation determine many actions of a polysaccharide on metabolism. Using traditional bacteriological techniques, which require isolation and growth of individual bacteria, human colonic microflora have been estimated to contain over 400 different dominant species [90]; however, new molecular techniques that measure bacterial DNA and rRNA [91–93] are revealing that the majority of bacterial groups have never been cultured and much of the bacterial DNA cannot be accounted for by known species. This means that much of our perceived knowledge of the flora may be inaccurate and should be reviewed.

The mainly anaerobic bacteria in the human colon use the carbohydrates to produce SCFAs (mostly acetic, propionic, and butyric acids) and the gases CO₂, H₂, CH₄, and H₂S. Colonic pH is also reduced with an increase in fermentation, but this is buffered by bicarbonate, which is exchanged for the SCFAs as they are absorbed. The fermentability of each carbohydrate depends on its structure and water solubility. The presence of bacteria with the required enzymes for each step of carbohydrate degradation is also essential. The degradation and fermentation of a complex carbohydrate structure may be the result of a synergy between several bacterial species [94], and some of the enzymes may take time to be expressed either by induction of enzymes, in originally predominant bacteria, or by an increase in dominance by new groups. It has been shown in rats that it can take many weeks before the colonic microflora are truly adapted to the introduction of a new gum into the diet [95].

The extent and rate of fermentation of nondigestible carbohydrates are important. The rate of fermentation may determine the site of SCFA production. Most colonic disease occurs in the distal colon, yet most fermentation occurs in the proximal colon; therefore, nondigestible carbohydrates that are slowly fermented may encourage prolonged fermentation and SCFAs at more distal sites. This effect may also be achieved by mixing easily and poorly
fermented carbohydrates in the same preparation. The fermentation of individual carbohydrates differs in the rate, extent, and pattern of SCFAs produced [96–98]. Pectin increases the proportion of acetic acid produced, guar gum and ispaghula tend to promote the production of propionic acid, and resistant starch, oat gum, and fructooligosaccharides increase the production of butyric acid [98].

Normal diet and more recently produced therapeutic feeds are more likely to contain mixtures of nondigestible carbohydrates. The fermentation of mixtures may not be predicted from studying the isolated carbohydrates. Moreover, slowly fermented carbohydrates may push the fermentation of more easily degraded sources, which produce butyrate, toward the distal colon, where they could have a greater impact on diseased tissue. A delay in fermentation (breath-hydrogen production) was seen when ispaghula and lactulose were fed together to human subjects [99]. The fermentation of resistant starch was shifted more distally by wheat bran (in pigs) and ispaghula (in rats) and both increased the fecal excretion of SCFAs [100,101].

8.2.9 Health Impact of Increased Colonic Fermentation

8.2.9.1 Colonic pH

The colonic fermentation of carbohydrate can result in a substantial fall in colonic luminal pH. The several benefits of reduced colonic pH include the inhibition of undesirable bacterial activities such as 7α dehydroxylase [102], which forms secondary bile acids that can act as cocarcinogens. Reduced colonic pH can also lead to precipitation of fatty acids, sterols, and other molecules that have been implicated as procarcinogens. Low pH reduces the rate of colonic cell proliferation, thought to be a risk factor for colon cancer [102]; however, the SCFAs produced during fermentation can stimulate cell proliferation [103], so under normal conditions the effects of pH here may be cancelled out. Nevertheless, populations in South Africa with a low risk of colonic cancer also have a lower colonic and fecal pH than those from high-risk populations [104].

8.2.9.2 Short-Chain Fatty Acids

Short-chain fatty acids have many potential actions that, in general, are beneficial for health. They promote the absorption of water and electrolytes [105] and inhibit the growth of pathogens [106], thus reducing the risk of diarrhea. They stimulate cell proliferation throughout the gut even though they are produced in the large intestine [107], and this may be important for wound healing after gut surgery [108] or after gastrointestinal disease. They may also modulate colonic motility [109], but it is unclear whether their effects result
in a speeding up or a reduction in colonic transit time. SCFAs tend to stimulate contractions of the terminal ileum [110,111], preventing back flow from the colon, and propionic and butyric acids inhibited contractions in the rat colon [112]. Antibiotics that reduce bacterial metabolism have been found to cause an increase in motility [113]. In addition to these general effects, each fatty acid has its own idiosyncratic actions.

8.2.9.3 Actions of Individual SCFA

8.2.9.3.1 Acetic Acid
Acetic acid is always the main SCFA produced, usually making up 50 to 70% of total SCFAs [114]. The action of acetate differs from that of other SCFAs in several ways. Acetic acid is the only SCFA to reach the systemic circulation in significant amounts [115]. It can thus provide energy for muscles and other tissues and is noninsulinogenic. It also has less effect on colonic motility than other SCFAs [116] and is the least preferred SCFA as an energy source for colonic enterocytes [117].

8.2.9.3.2 Propionic Acid
Propionic acid is usually the second most abundant SCFA produced during fermentation. Propionic acid is absorbed into the portal vein but is mainly removed by the liver [115]. It is gluconeogenic, unlike the other SCFAs, and may inhibit cholesterol synthesis by the liver [118] and influence insulin sensitivity [119]. Although this effect has been well demonstrated under experimental conditions, it is not clear if enough propionic acid is formed in the colon and absorbed into the portal vein to influence cholesterol synthesis [120]. It is worth noting, however, that carbohydrates associated with cholesterol-lowering effects are often those that promote propionate production, and a decrease in the ratio of acetate to propionate production appears to be related to a lower hepatic synthesis of cholesterol from acetate [121].

8.2.9.3.3 Butyrate
It has long since been established that butyric acid is the preferred fuel for the colonic enterocyte [117]. More recent interest has centered on the many potential anticancer properties of butyrate. An increase in the rate of colonic cell proliferation is believed to be a risk factor for cancer. On the other hand, all cells in the colon are programmed with a time to die (programmed cell death, or apoptosis). It is probably the balance between the levels of proliferation and apoptosis that determines whether a cancer will or will not develop. Butyrate has been shown to stimulate cell proliferation of normal colonic cells and to stimulate apoptosis in cancer cells in vitro [122]. Butyrate promotes cell differentiation of cancer cells [123,124] and inhibits histone deacetylase, which may promote DNA repair [125,126]. Butyrate has also been shown to heal damaged mucosa in inflammatory bowel disease, such as ulcerative colitis [127].
8.2.9.4 Effect of Nondigestible Carbohydrates on Other Bacterial Activity or Metabolic Processes

Colonic bacteria are capable of a wide range of metabolic activities. Increasing bacterial numbers by providing fermentable energy sources may influence the transformation of drugs [128], the production or disposition of toxins and carcinogens [129,130], and the production of ammonia [131,132], nitrogen cycling [133], and other activities. The main bacterial enzymes involved in drug and other xenobiotic transformations are azoreductase, β-glucosidase, β-glucuronidase, and nitroreductase. The effect of nondigestible carbohydrate on the activities of these enzymes is variable and has been extensively reviewed elsewhere [129], but, in general, fermentable nondigestible carbohydrates are likely to increase their activity. The relevance of this enzyme activity to the causes of human disease is not clear, but several animal studies suggest that some types of fiber may protect against intestinal tumors induced by chemical carcinogens [134], while others suggest little effect or that carcinogenesis may even be promoted [136].

The nature of nondigestible carbohydrates appears to be an important factor in determining their effect on carcinogenesis, but conflicting results have been reported for the same types of fiber; for example, konjac mannan has been shown to inhibit 1,2-dimethylhydrazine-induced carcinogenesis in rat colon. Also, although its consumption did not lead to significant changes in microflora composition, a significant reduction in fecal β-glucuronidase, nitroreductase, and azoreductase activity was observed in F344 rats [134], as well as a reduction in β-glucuronidase and nitroreductase activity in C3H/He rats [135]. Putrefactive metabolic products (phenol, indole, and p-cresol) are also significantly reduced [135]. Other soluble nonstarch polysaccharides such as pectin and guar gum have been shown to enhance chemically induced colon cancer [137]; however, much of the data from animal studies arise from examination of different stages of carcinogenesis, which makes interpretation and comparison of results difficult. Indeed, a protective effect has been demonstrated for pectin during the tumor-promotion stage [138]. The ability of certain nondigestible carbohydrates to stimulate the fecal excretion of carcinogens differs significantly.

A recent study by Ferguson et al. [130] found that lignified plant cell wall material was extremely effective in eliminating 2-amino-3-methylimidazo[4,5-f]quinoline (IQ) from rat plasma; in contrast, resistant starch and soluble fiber appeared to increase the bioavailability of the same carcinogen. Harris et al. [139] demonstrated a protective effect against carcinogenesis by the consumption of insoluble wheat bran in an animal model, a finding that has been supported by some human intervention studies [140–142]. Fibers consumed as intact plant cell material may have a greater effect on carcinogenesis because they have a greater ability to absorb carcinogens [139] and also contain a
number of phytochemicals with known protective properties against cancer as well as coronary heart disease (CHD) and other chronic disease [143]. Thus, many factors, including the physicochemical properties of the fiber, the amount ingested, and the composition of the colonic microflora, may play an important role in colon carcinogenesis.

Bacteria also have a major role in deconjugating and dehydroxylating bile acids. This may influence the composition of bile and, hence, gallstone formation and cholesterol metabolism. Secondary bile acids are produced on deconjugation and are known to promote tumors in animal colon [144]. Additionally, they stimulate colonic secretion [145,146] and motility, as do hydroxy fatty acids, which are also produced by colonic bacteria [147,148].

The colon is a major site of nitrogen cycling. Nitrogen is a growth factor for colonic bacteria, and a certain amount is obtained from undigested dietary protein entering the colon. In addition, pancreatic and intestinal secretions and sloughed epithelial cells can be utilized, but the major source of nitrogen is blood urea, which diffuses into the lumen [149,150]. Fermentable carbohydrate in the colon will increase the absorptive mucosal surface area. Blood flow to the cecum is also increased, which allows greater diffusion of urea from the bloodstream to the lumen. The bacteria convert urea to ammonia, which can be reused by the bacteria to produce protein, which can be excreted in the feces [151,152] with a net result of a shift in nitrogen excretion from urine to feces. Lactulose has been used for some time to reduce colonic ammonia absorption in hepatic encephalopathy.

The extent to which nitrogen excretion is affected by a nondigestible carbohydrate is dependent on its structural or physicochemical nature; for example, the consumption of fructo- and xylooligosaccharides has a greater effect on the depression of ammonia absorption from the colon and on increased fecal nitrogen excretion than gum arabic and cellulosic oat fiber in the rat [132], an effect that may be related to pH. Although Ohta et al. [133] reported increased fecal nitrogen excretion after consumption of fructooligosaccharide in the rat, this effect was not dose dependent and did not differ with polymer chain length. It is clear that nondigestible carbohydrate plays an important role in nitrogen balance, and that the strain on renal function may be significantly reduced by increasing fecal nitrogen excretion. This is of major significance given the recent trend of consuming low-carbohydrate diets for weight loss.

8.2.10 Prebiotic Effects of Nondigestible Carbohydrates

There has been increasing interest in the role of certain nondigestible carbohydrates in promoting the growth of so-called “healthy” lactic acid bacteria at the expense of less desirable species in the colon. These “prebiotic” actions
are mainly attributed to the resistant oligosaccharides but have also been demonstrated for resistant starch [153]. Both of these also increase colonic butyrate production as discussed above.

Ideally, prebiotic carbohydrates should selectively stimulate the growth and activity of a limited number of beneficial bacterial species already present in the human gut [154]. These bacteria (predominantly bifidobacteria and lactobacilli) are believed to confer health benefits similar to those seen with commercial probiotic organisms [155,156].

The popularity of these prebiotics as functional food ingredients has increased rapidly, particularly in Japan and more recently in Europe. The Japanese government legislated in 1991 for fructooligosaccharides, galactooligosaccharides, soybean, and palatinose oligosaccharides to be included in “foods for specified health use” (FOSHU), and by 1996 34 new approved food products contained oligosaccharides as the functional food ingredient to maintain a good gastrointestinal environment [8]. By definition, nondigestible oligosaccharides remain intact within food and through the upper gastrointestinal tract, so, unlike probiotic bacteria, there is no doubt that they reach the colon, where they may affect the microflora [157].

The in vivo effects of nondigestible oligosaccharides on the intestinal microflora have been investigated in animals [133,158–160] and humans in both health [3,4,154,161,162] and disease [163,164]. Short-chain fructooligosaccharides in particular have been shown to preferentially stimulate the growth of Bifidobacteria spp. in the human colon [3,154,165]. Bifidobacteria are believed to have relatively high amounts of β-fructosidase, which, is selective for the β-[1-2] bonds present in fructooligosaccharide. Gibson et al. [154] reported that selective fermentation of 15 g/day fructooligosaccharide resulted in a significant increase in the numbers of healthy bacteria in the colon and a decrease in the numbers of potential pathogens such as clostridia and fusobacteria. Contrary to this, Yamada et al. [166] found fructooligosaccharide to be a good substrate for the growth of Clostridium and Bacteroides spp. Nevertheless, a reduction in the numbers of pathogenic bacteria in the colon after fructooligosaccharide consumption has been demonstrated [167,168]. Rao [3] showed that ingestion of 5 g/day fructooligosaccharide for 11 days resulted in a nearly 1 log increase in bifidobacteria numbers relative to a sucrose placebo. This increase was greatest in subjects who had lower initial bifidobacteria counts, thus showing that prebiotics may work best for those with lower populations in the first place. In addition to their other effects, bifidobacteria may secrete a bacteriocin-type substance that is active against clostridia, Escherichia coli, and many other pathogenic bacteria [167].

Other potential prebiotic nondigestible oligosaccharides are galacto- and xylooligosaccharides [161–163,169–172], although there is less evidence for their effects than for fructooligosaccharides. In healthy human subjects, 10 g/day transgalactooligosaccharides for 21 days significantly increased fecal
bifidobacteria counts [162]. Alles et al. [163], however, reported no beneficial change in the composition of human intestinal microflora in vivo after consumption of transgalactooligosaccharides (7.5 or 15 g/day) for the same time period. Administration of 5 g/day xyloooligosaccharide for 3 weeks [161] increased intestinal bifidobacteria counts from around 10% to 32% in human subjects. Fecal acetic acid concentration was also increased and was associated with a lower fecal pH. In vitro mixed-culture studies [170] have shown that the largest increases in populations of bifidobacteria have occurred with xyloooligosaccharide and lactulose. Lactobacilli populations were increased the most in the presence of fructooligosaccharide, and glucooligosaccharide was the most effective in reducing numbers of clostridia. In human-flora-associated (HFA) rats, fructooligosaccharides and galactooligosaccharides increased bifidobacteria numbers by 2 log values compared with glucooligosaccharide [160]. This was associated with a similar decrease in pH and an increase in total SCFA concentration for all three oligosaccharides compared to controls; however, the SCFA profile, bacterial enzyme activity, and gas production differed among oligosaccharide types. The effects of prebiotics are short lived, as bifidobacteria counts return to preingestion levels if the supplement is stopped [3,161].

Current knowledge of the oligosaccharide content of human milk [5] and the role of oligosaccharides in the proliferation of bifidobacteria has led to increased interest in the use of prebiotic oligosaccharides in infant formula in Europe to produce a bacterial flora more like that of the breast-fed infant [173]. The evidence supporting the benefits of prebiotic oligosaccharides in infant formula is, however, limited at present [173–175], although significant increases in fecal bifidobacteria populations have been shown [175].

The concept of “synbiotics” combines the effects of both pre- and probiotics [167]. Their use should have a synergistic effect rather than just an addition of the effects of the prebiotics and probiotics. At present, few reports have been published regarding the use of synbiotics by humans. Attempts to stimulate the survival and persistence of commercial probiotics by the addition of galactooligosaccharide to yogurt have been met with limited success [176], and further work is required in this area. Increasing numbers of studies, however, are now underway that explore the actions of synbiotics in diseases such as inflammatory bowel disease and cancer prevention.

8.2.11 STOOL OUTPUT

Nondigestible carbohydrates may increase stool output but the mechanisms by which they act are several and are affected by fermentation and other factors. The most effective stool bulkers are those that are poorly fermentable. Here, the major mechanism is their water-holding capacity (WHC); however, the water-holding capacity of a polysaccharide before ingestion is a poor indicator of its action on stool output because most polysaccharides with large WHCs are readily fermented [177]. The WHC after fermentation in vitro is a better
indicator, but even this will sometimes give misleading results [178]. Many subjects need to be studied to provide a better index of the action of a particular polysaccharide in a population, but these results do provide a useful screen when evaluating potential food or pharmaceutical products.

If a nondigestible carbohydrate is fermented, bacterial numbers may increase, and this in itself could increase fecal bulk [179], as bacteria are mainly composed of water; however, most readily fermented fibers are poor stool bulkers. The impact of fermentation in stool bulking action can be seen when wheat bran is ingested with and without antibiotics [180]. The action on stool output is much increased when antibiotics are used. The effects of fermentation products on motility are discussed above.

Many other factors determine stool output, apart from bacterial fermentation. Other actions of nondigestible carbohydrate are important — for example, the effects on transit time, stool consistency, and frequency. Edwards and Eastwood [181] studied the action of wheat bran in comparison with ispaghula (a commonly used anticonstipation agent) on stool output in rat. Wheat bran was partially fermented in the cecum but had no effect on the contents of the whole colon, either in terms of volume or percentage water. Ispaghula, on the other hand, was fermented throughout the colon and increased the volume of the entire colon, suggesting that wheat bran increased propulsion, whereas ispaghula increased colonic bulk. It has been shown that plastic particles can be as effective as wheat bran in increasing stool output and it may be that insoluble particulate fibers act by stimulation of mechanoreceptors inducing propulsive motor activity [182–184]. If stool output is increased substantially, then several nutritional consequences result, including increased losses of energy, nitrogen, water, and electrolytes. Too little stool output, however, results in constipation and increases the risk of diverticulosis and colon cancer.

8.2.12 **Nondigestible Carbohydrate in Therapeutic Enteral Diets**

Therapeutic enteral diets must be liquid to allow administration through tubes directly into the gastrointestinal tract and to afford easy absorption of nutrients. Because many nonstarch polysaccharides are either highly viscous or insoluble, their incorporation into enteral feeds is somewhat problematic. Thus, therapeutic elemental or polymeric diets have, until recently, contained no source of dietary fiber. This has resulted in patients, including children, on enteral feeds suffering constipation and atrophy of the colonic mucosa. Research into the inclusion of hydrolyzed nondigestible carbohydrates with low viscosity in such feeds is increasing [184–188], and many liquid diets now contain carbohydrates such as soy polysaccharide, pectin, and carboxymethylcellulose. The inclusion of partially hydrolyzed guar gum in enteral nutrition has reduced the incidence of both diarrhea in sepsis patients [187,188] and constipation in the elderly [186].
8.2.13 Potential Adverse Effects of Nondigestible Carbohydrates

In contrast to the proposed health benefits, the consumption of some nondigestible carbohydrates may lead to adverse effects such as flatulence, bloating, and abdominal discomfort [4]. This has been attributed to excess gas production, particularly when high doses are consumed or when the oligosaccharide chain length is long [189,190]. According to Bouknik et al. [4], 10 g/day of short-chain fructooligosaccharide is the optimal and well-tolerated dose. The incidence of these side effects of fermentation may reduce the compliance of high-fiber diets, but they often subside with prolonged use of the nondigestible carbohydrate.

As discussed earlier, the effects of fermentable dietary fiber on cell proliferation in the large intestine and the repeated demonstration of increased tumor yield in animal models of colon cancer with some fermentable fibers have given rise to criticism of the recommendation to increase fermentable fiber intake [191]. The increasing knowledge regarding the benefits of butyrate, however, and the difficulties in extrapolating from acute studies using high-dose carcinogens in rats to the sporadic cancers seen in humans have resulted in a general acceptance that dietary fiber recommendations are still appropriate.

One major area of concern for the potential adverse effects of a high-fiber diet has been in the diet of rapidly growing infants and children. Very little research has been carried out in young children, and this has led to recommendations for daily fiber intake in these age groups being based on guesswork, caution, and extrapolation of data from adults. The U.K. dietary recommended values (DRVs) for children state that fiber intake should be proportionately lower than that of adults and related to body size and that children under 2 years of age should not take dietary-fiber-containing foods at the expense of energy-rich foods [192]. In the United States, suggested levels include the child’s age (in years) + 5 g/day [193,194] or 0.5 g/kg [195].

It has been feared that a diet too high in dietary fiber may lead to growth retardation and malabsorption of minerals [192]. An old study [196] on vegetarian children did find lower growth velocities in children under 2 years old, but this finding may have been related to delayed weaning or the lack of meat, rather than the dietary fiber content of the diet. Vegan children are reported to be shorter than normal controls, but this may not be related to dietary fiber intake [197]. There have been other reports of growth delay in vegetarian children, and children on macrobiotic diets [198], but it is difficult to assess these for the impact of the influence of dietary fiber, as these diets have many other variations from normal.

A high-fiber, low-fat diet in Scottish school children 7 to 8 years old was found to have little effect on growth [199]; no association between growth and the higher intake of fiber was observed. With the growing risk of obesity in
children, a fiber-rich diet that reduces energy intake may be of great benefit [200]. Fiber consumption was associated with a reduced risk of overweight in a group of 445 Native Canadian children (2 to 19 years old) with a high risk of obesity [201]. For each 0.77-g/MJ increase in fiber consumption in the previous 24 hours of the study day, a 1.4-fold decrease in the risk of overweight resulted. No relationship with other macronutrients was found. Thus, a high-fiber diet did not seem to adversely affect growth but instead may reduce the risk of being obese.

8.3 CONCLUSION

In conclusion, the Western diet has changed considerably over recent years, with a move away from natural plant foods toward the consumption of greater amounts of more heavily processed or convenience foods. As a consequence, many individuals consume diets that contain novel and isolated nondigestible carbohydrates added by food manufacturers as preservatives, to add new or improved textural attributes, or to serve as functional food ingredients, such as prebiotics, for proposed health benefits. Although some potential negative effects have been suggested, it is generally accepted that nondigestible carbohydrates have many beneficial actions that prevent the development of diseases such as coronary heart disease and cancer. Increased understanding of the very different physiological roles and mechanisms of action of soluble and insoluble fiber highlights the need for a diet that contains a diverse range of nondigestible carbohydrate for health. This advice should be further developed, however, to take into account that recent epidemiology has emphasized the role of whole grains rather than dietary fiber in preventing chronic disease. Thus, the health benefits may not only be the result of the structure and functionality of the carbohydrate but are almost certainly related to associated compounds such as phytochemicals and vitamins.

REFERENCES


38 Ellis, P.R., Roberts, F.G., Low, A.G., and Morgan, L.M., The effects of high-
molecular-weight guar gum on net and apparent glucose absorption and net and
apparent insulin and gastric inhibition of polypeptide production in the growing
pig: relationship to rheological changes in jejunal digesta, *Br. J. Nutr.*, 74,
39. Higham, S.E. and Read, N.W., The effect of ingestion of guar gum on ileostomy
40. Blackburn, N.A. and Johnson, I.T., The effects of guar gum on the viscosity of
the gastrointestinal contents and on glucose uptake from perfused jejunum of the
gum on the physical properties of gastrointestinal contents and on the uptake of
D-galactose and cholesterol by rat small intestine *in vitro*, *Br. J. Nutr.*, 62,
42. Cameron-Smith, D., Collier, G.R., and O’Dea, K., Effects of soluble dietary fibre
on the viscosity of gastrointestinal contents and the acute glycaemic response in
43. Favier, M.-L., Bost, P.-E., Guittard, C., Demigné, C., and Rémésy, C., Reciprocal
influence of fermentations and bile acid excretion on cholesterol-lowering effect
44. Gallaher, D.D., Hassel, C.A., Lee, K.J., and Gallaher, C.M., Viscosity and fer-
mentability as attributes of dietary fiber responsible for the hypocholesterolemic
45. Levrat-Verney, M.-A., Behr, S., Mustad, V., Rémésy, C., and Demigné, C., Low
levels of viscous hydrocolloids lower plasma cholesterol in rats primarily by
intestinal contents viscosity leads to greater excretion of neutral steroids but not
47. Ide, T., Moruichi, H., and Nihimoto, K., Hypolipidemic effects of guar gum and
supplemented with depolymerised guar gum reduces plasma cholesterol concen-
50. Leclère, C.J., Champ, M., Bulliot, J., Guille, G., Lecannu, G. et al., Role of
51. Blackburn, N.A., Holgate, A.M., and Read, N.W., Does guar gum improve post-
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9.1 INTRODUCTION

Starch is a major reserve polysaccharide of green plants and probably the second most abundant carbohydrate in nature next to cellulose. The pure material can be obtained by simple processes from various plant sources such as seeds, stems, and roots. Starch is convertible to many useful materials by chemical and biochemical techniques, as well as by fermentation; therefore, the structures, properties, and utilization of this polysaccharide have been the subject of many investigations since the beginning of modern chemistry and biochemistry. Although starch seems to be a simple material because it is comprised of only glucose, it is not simple, and we do not yet fully understand even its primary structure.

In the 1930s, starch was thought to be a heterogeneous material, and many attempts were made to separate and characterize its components, but these were not successful. The breakthrough was achieved by Meyer and his coworkers [1] in 1940. They extracted a new material from maize starch with hot water at a temperature (70 to 80°C) slightly above gelatinization. The extract, although minor in amount, had different properties from the maize starch. It was comprised of a long linear molecule linking about 300 glucosyl residues,
as determined by methylation, end-group analyses, degradation with β-amylase, and osmotic pressure measurement. This unique linear material was defined as amylose and the remainder, branched molecules, as amylopectin. The terms “amylose” and “amylopectin” had both been used before that time without clear structural evidence and had even been reversed, thereby causing confusion. Although amylose and amylopectin were not quantitatively separated and the remainder of the hot-water extract still contained amylose and amylopectin, the discovery of this linear molecule was a landmark in the study of starch. Soon after the discovery of amylose, Cori and Cori [2] synthesized this linear molecule in vitro using muscle phosphorylase and also synthesized glycogen by taking advantage of cooperative action with branching enzymes. This was the first successful synthesis of a biopolymer in vitro. Quantitative separation of amylose and amylopectin was first carried out by Schoch [3], who succeeded in crystallizing amylose (referred to as the A fraction) as an inclusion complex with 1-butanol-amylose from a fully dispersed, hot starch solution by cooling, and amylopectin (the B fraction) was recovered from the mother liquor.

Although amylose was originally defined as being linear, it is now well recognized that some amylose molecules have several branches, such as in amylopectin. In addition, the presence of materials intermediate between amylose and amylopectin has been suggested in amylomaize and wrinkled-pea starches. The properties of these starch components are summarized in Table 9.1. To characterize the branched structures of these molecules it is necessary to determine the number and location of the branch linkages in a molecule. Amylolytic enzymes with well-defined specificities are useful for

<table>
<thead>
<tr>
<th>Property</th>
<th>Amylose</th>
<th>Linear</th>
<th>Branched</th>
<th>Amylopectin</th>
<th>Intermediatea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Branch linkage (%)</td>
<td>0.2–0.7</td>
<td>0</td>
<td>0.2–1.2b</td>
<td>4.0–5.5</td>
<td>2–3.5</td>
</tr>
<tr>
<td>$\bar{C}_{\text{T}}$</td>
<td>100–550</td>
<td>800a</td>
<td>140–250b</td>
<td>18–25</td>
<td>30–50</td>
</tr>
<tr>
<td>$\bar{M}_{\text{p}}$</td>
<td>700–5000</td>
<td>105–104</td>
<td>105–104</td>
<td>105–104</td>
<td>105–104</td>
</tr>
<tr>
<td>$\lambda_{\text{max}}$ (nm)</td>
<td>640–660</td>
<td>—</td>
<td>—</td>
<td>530–570</td>
<td>570–580</td>
</tr>
<tr>
<td>Blue value</td>
<td>1.2–1.6</td>
<td>—</td>
<td>—</td>
<td>0.3–0.8</td>
<td>0.7</td>
</tr>
<tr>
<td>Iodine affinity (g/100 g)</td>
<td>19–20.5</td>
<td>—</td>
<td>—</td>
<td>0–1.2</td>
<td>2–10</td>
</tr>
<tr>
<td>Helix formationc</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>β-Amylosis limit (%)</td>
<td>70–95</td>
<td>100</td>
<td>40</td>
<td>55–60</td>
<td>57–75</td>
</tr>
</tbody>
</table>

a Amylomaize amylopectin.  
b Rice amylose.  
c With 1-BuOH.
this purpose because conventional chemical and physiochemical methods have limitations. In this chapter, the structures of starch components and granules are described from an analytical aspect, and the focus is on the use of enzymic methods.

9.2 ACTIONS OF STARCH-DEGRADING ENZYMES

Starch-degrading enzymes, such as amylases, debranching enzymes, and phosphorylase, are useful not only for the analysis of fine structures of starch molecules but also for the production of glucose, maltose, various oligosaccharides, and modified starches. In this section, the actions of these enzymes are briefly described. Comprehensive reviews have been published on amylases [4–7].

9.2.1 (1→4)-α-LINKAGE-_SPLITTING ENZYMES

9.2.1.1 α-Amylase

α-Amylases (enzyme class EC3.2.1.1) are commonly found in animals, plants, and microbes. They hydrolyze starch endwise at inner (1→4)-α-linkages and rapidly reduce viscosity and iodine coloration (blue value) with a gradual increase in reducing value. The products have an α-configuration. The percent decrease of blue value vs. hydrolysis is rapid but characteristic of origins. Some α-amylases, especially Aspergillus oryzae (Taka-α-amylase A), show a randomness of hydrolysis similar to that of 1-M H₂SO₄ [8]. Achroic points, where the iodine coloration disappears, are 13 to 16% hydrolysis for Bacillus subtilis liquefying α-amylase and 20 to 30% for A. oryzae (Taka-α-amylase A), saliva, pancreas, and B. subtilis saccharifying α-amylase. The (1→6)-α-linkages and some neighboring (1→4)-α-linkages, depending on their specifications, cannot be split, and all the branch linkages remain as branched oligosaccharides; however, the α-amylase from Thermoactinomyces vulgaris has been found to weakly hydrolyze (1→6)-α-linkages [9]. The ester phosphate groups at C-6 and C-3 of amylopectin do not allow the enzymes to accommodate the phosphorylated residues at their active sites, and phosphorylated oligosaccharides remain in the final products [10]. The smallest branched oligosaccharides and phosphorylated oligosaccharides produced from amylopectin by some α-amylases are shown in Table 9.2. Some α-amylases appear to also act as transferases because it has been found that they transfer glucosyl residue from α-D-glucosyl-glucosyl fluoride to C-4 of glucose or high maltooligosaccharides and produce higher oligosaccharides [11].

Human pancreatic and salivary α-amylases show very similar actions. Both amylases produce G₂ preferentially from reducing residues of G₃, G₄, and G₅, and essentially do not act on G₃; however, saliva enzymes hydrolyze G₃ mainly
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into G₂ and G₅, and pancreatic enzymes hydrolyze G₇ mainly into G₃ and G₄, similar to porcine pancreatic enzyme [12]. Porcine pancreatic α-amylase hydrolyzes linear amylose, mainly into G₂ and G₃ and finally into G₁ and G₂ after prolonged incubation with a large amount of the enzyme. Glucose is preferentially liberated from the reducing residue of maltotriose [13]. The enzyme hydrolyzes adjacent and penultimate (1→4)-α-linkages toward the nonreducing side and the third linkage toward the reducing side at C-6 branched residues. Kainuma and French [14,15] identified several branched oligosaccharides having single to triple (1→6)-α-linkages in the limit hydrolyzate of waxy cornstarch.

Cereal grains, such as barley, wheat, rye, and rice, have none or very low levels of α-amylase, but they rapidly increase their activity during the germination period. The malted-rye [16] and barley [17] enzymes hydrolyze amylose mainly to G₂ and G₄ to G₇, then higher oligosaccharides are finally degraded to a large amount of G₂ and small amounts of G₁ and G₃. Two isozymes, AMY-1 and AMY-2, have been identified in germinated barley; AMY-1 is produced during the maturation process of grains, and AMY-2 is synthesized on germination. Their actions on linear maltodextrins have been investigated [18]. These two isoenzymes show similar actions and hydrolyze mainly or almost exclusively G₄ into G₂, G₅ into G₂ and G₃, G₆ into G₂ and

<table>
<thead>
<tr>
<th>α-Amylase</th>
<th>Smallest Branched Oligosaccharide</th>
<th>Smallest Phosphorylated Oligosaccharide</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. subtilis liquefying</td>
<td><img src="image1" alt="Diagram" /></td>
<td><img src="image2" alt="Diagram" /></td>
</tr>
<tr>
<td>B. subtilis saccharifying</td>
<td><img src="image3" alt="Diagram" /></td>
<td><img src="image4" alt="Diagram" /></td>
</tr>
<tr>
<td>Taka-amylase A</td>
<td><img src="image5" alt="Diagram" /></td>
<td><img src="image6" alt="Diagram" /></td>
</tr>
<tr>
<td>Hog pancreas</td>
<td><img src="image7" alt="Diagram" /></td>
<td><img src="image8" alt="Diagram" /></td>
</tr>
</tbody>
</table>

*Note:* ○, glucose; —, (1→4)-α-linkage; ↓,(1→6)-α-linkage; P₃ and P₆, phosphate ester at C-3 and C-6, respectively.

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G₄, G₇ into G₁ and G₆, G₈ into G₂ and G₆, and G₉ into G₂ and G₇. They appear to form G₁ more than other α-amylases; however, AMY-1 and AMY-2 show slightly different actions. For example, AMY-1 hydrolyzes mainly G₁₀ into G₄ and G₇, and AMY-2 hydrolyzes it into G₂ and G₈. They appear to have 10 subsites, with the catalytic site located between subsites 7 and 8 [19]. Another difference is that AMY-1 is active on raw-starch granules.

Taka-amylase A completely hydrolyzes amylose to G₁ and G₂. Oligosaccharides higher than G₄ are readily hydrolyzed unlike G₂ and G₃, especially G₂ [20,21]. This enzyme also hydrolyzes cyclodextrins in the order of α- < β- < γ-cyclodextrin [22].

Liquefying α-amylase of Bacillus subtilis or B. amyloliquefacience has been observed to hydrolyze amylose to a mixture of G₁–G₆, preferentially G₂, G₃, and G₆. It liberates G₁ from G₆ by attacking at the reducing side [23,24] and has a weak action on G₅ but almost no action below G₅ [23]. The limit of hydrolysis of starch was approximately 35%. This enzyme does not readily attack the frequently branched portion of amylpectin. It easily liberates G₆ from outer chains of amylpectin [22,23] and cuts the linear portions of linking clusters of amylpectin and β-limit dextrin (β-LD) to produce large, branched molecules, G₃ and G₁ [23–27]. Thus, the enzyme showed a dual specificity for the formation of G₁ and G₆. The enzyme has a relatively strong ability to attack starch granules. Bertoft et al. [28] reported that α-amylase of B. amyloliquefacience attacked raw wrinkled-pea starch and preferentially hydrolyzed amylose to small linear dextrins (degree of polymerization [DP] 2 to 100). It also attacked the branched molecules of the granule without solubilization. Raw potato starch is least susceptible to amylases, but B. circulans [29] and B. subtilis 65 [30] produced α-amylases with strong activity for raw potato starch.

The saccharifying α-amylase from Bacillus subtilis is useful for the structural analysis of amylpectin and glycogen because it has been shown to specifically hydrolyze (1→4)-α-linkages at the nonreducing side of a branch linkage producing 6′-glucosyl maltriose [31]. It hydrolyzes amylose to G₁, G₃, and G₅, and finally G₁ to G₂ and G₃. Streptococcus bovis α-amylase showed a similar action [32]. From the characterization and quantitative determination of singly to triply branched oligosaccharides in the limit hydrolyzate of the β-limit dextrin of waxy rice starch with bacterial saccharifying α-amylase, the frequencies of one, two, and more than two (1→4)-α-linkages apart between adjacent branch linkages were calculated as 0.4, 20.8, and 78.8%, respectively [33]. The structure and yield of these oligosaccharides are shown in Table 9.3. Xanthomonas campestris K-11151 α-amylase produces G₁ from G₂. This is mainly due to the production of G₂ by the condensation of G₂ molecules followed by the hydrolysis of G₄ to G₃ and G₁ [38a]. Oligosaccharides serve as good substrates for the condensation reaction in this enzyme. One must pay attention to reactions of this kind when the substrate specificity of a certain enzyme is being evaluated.

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9.2.1.2 Isopullulanase and Neopullulanase

The (1→4)-α-linkages of pullulan are specifically hydrolyzed by two enzymes, isopullulanase (EC3.2.1.57) and neopullulanase. *Aspergillus niger* produces isopullulanase [34], which hydrolyzes (1→4)-α-linkages of the nonreducing sides of the maltotriosyl residues and yields isopanose, as shown in Figure 9.1. The enzymes from *Thermoactinomyces vulgaris* [35], *Bacillus stearothermophilus* [36], *B. licheniformis* [37], and *Xanthomonas campestris* K-11151 [38] hydrolyze (1→4)-α-linkages of the reducing side of the maltotriosyl residue and produce panose. This is the typical activity of neopullulanase. These

<table>
<thead>
<tr>
<th>Structure</th>
<th>Yield</th>
<th>Structure</th>
<th>Yield</th>
</tr>
</thead>
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<td><img src="structure8.png" alt="Structure" /></td>
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<td><img src="structure12.png" alt="Structure" /></td>
<td>0.5</td>
</tr>
</tbody>
</table>

* Relative yield by mole.

**Note:** For key, see Table 9.2.

**Source:** Adapted from Umeki, K. and Yamamoto, T., *J. Biochem.*, 78, 897, 1975.
Carbohydrates in Food

Enzymes are useful for the structural analysis of branched oligosaccharides produced by the actions of various α-amylases from amylpectin and glycogen.

9.2.1.3 β-Amylase

β-Amylase (EC3.2.1.2; 1,4-α-D-glucan maltohydrolase) has been abundantly found in plants, especially wheat, soybean, and sweet potato, and in some bacterial cultures (Bacillus polymyxa, B. cereus, and B. megaterium) [39]. The crystalline enzyme has been prepared by rather simple processes from sweet potato [40], including recrystallization from ammonium sulfate solution for long storage to protect from microbial contamination. Commercial enzymes from sweet potato may contain α-glucosidase, which can be removed by DEAE-Sephadex A-50 [41]. Amylose, amylpectin, and glycogen are hydrolyzed exowise by β-amylase at the second (1→4)-α-linkages from the nonreducing terminal residues until near the branch linkages, and β-maltose and β-LD are produced. The β-LD has been suggested to have two or three glucosyl residues on A chains and one or two glucosyl residues on the outer side of the branch linkages of the B chains, depending on the even or odd number of glucosyl residues on the outer chains [42], as shown in Figure 9.2. Therefore, the average outer-chain length (OCL) is 2. The β-LD is useful for the analysis of the branched nature of amylpectin and amylose because it reserves intact all the branch linkages of these polysaccharides. The linear amylose molecules are completely degraded into maltose if the molecules are composed of an even number of glucosyl residues, and one glucose molecule is produced at the final hydrolysis if they include an odd number of residues; however, maltotriose may remain because it is difficult to hydrolyze. The action on amylpectin appears to proceed in two stages: first, a rapid progress up to about 52% hydrolysis and, second, a slow progress to completion. In the first stage, A chains are shortened to maltotetraosyl residues, which are hydrolyzed to maltosyl residues in the second slow stage [43]. The slow hydrolysis can be overcome by using an excess amount of the enzyme.

FIGURE 9.1 Actions of (A) isopullulanase and (B) neopullulanase. ○, glucose; —, (1→4)-α-linkage; ↓, action site.
The enzyme cannot bypass the phosphate ester groups at C-6 or C-3, as the branch and phosphate esters on the outer chain are exposed near the nonreducing terminal residues of the β-LD (Figure 9.2) [44]. Either the outer chain from the site of C-6 phosphate was completely removed or one residue remained outside, depending on whether there was an even or odd number of outer-chain lengths. Plant β-amylase is unable to digest raw starch granules but bacterial enzymes are active [39]. The limits of hydrolysis are 54 to 61% for various amylopectins and 70 to 100% for amyloses, depending on the amount of branched molecules.

Because two or three and one or two glucosyl residues remain in the outside branch linkages of the A and B chains, respectively, in the β-LD of amylopectin, the average chain lengths of the outer chains (OCLs) and inner chains (ICLs) of amylopectin can be calculated from the β-amylolysis limit and the average chain length (CL) by Equation 9.1 and Equation 9.2:

\[ \text{OCL} = \frac{\text{CL} \times \beta\text{-amylolysis limit (\%)}}{100} + 2 \] (9.1)

\[ \text{ICL} = \text{CL} - \text{OCL} - 1 \text{ (branched residue)} \] (9.2)

### 9.2.1.4 Glucoamylase

Glucoamylase (EC3.2.1.3; 1,4-α-d-glucan glucohydrolase) is produced by many fungi and some bacteria. It is capable of hydrolyzing completely both
α-(1→4)- and (1→6)-α-linkages in starch and glycogen exowise from the nonreducing terminal residues, producing β-D-glucosyl-glucose. The (1→6)-α-linkage is hydrolyzed only after the main outer chain is completely removed. The branched glucosyl residues are not hydrolyzed easily, as in (1→4)-α-linked residues; therefore, hydrolysis of (1→6)-α-linkage is the rate-limiting step. Cyclodextrins are not hydrolyzed because they do not have a nonreducing end. Branched cyclodextrins having one or more (1→4)-α-glucan side chains are hydrolyzed at their side chains and produce glucosyl cyclodextrins. Esterified phosphate groups of amylopectin are barriers and terminate the action. The 6-phosphoryl groups are exposed at the nonreducing residue, and one glucosyl residue remains outside of the 3-phosphoryl groups after the extensive action of the enzyme [10,45]. Because amylopectin molecules contain small amounts of these ester phosphate groups, carefully purified enzyme is unable to hydrolyze starch completely, particularly potato starch. Glucoamylase hydrolyzes potato starches (359 to 915 ppm), potato amylopectin (1067 ppm), and waxy rice starch (16 ppm) by 83 to 88, 81, and 96%, respectively, and phosphorylated glucoamylase limit dextrin (γ-LD) remains [45]. The structure of γ-LD is shown in Figure 9.3. Some chains are completely hydrolyzed, some chains remain as glucosyl stubs, and linear or glucosylated chains are formed by subsequent debranching with isoamylase.

Commercial soluble starch is also not hydrolyzed completely because most soluble starches are produced from potato starch, and, in addition to phosphate groups, it contains carbonyl or carboxyl groups, which are formed during the solubilization process; however, some purified preparations of Rhizopus sp. have been reported to hydrolyze these starches completely due to the action of contaminated weak α-amylase [46], which bypassed phosphorylated residues.
and the other modified sites. Starch is suggested to be completely converted to glucose by assays of reducing power, but tiny amounts of phosphorylated oligosaccharides are found in the hydrolyzate.

The complete removal of α-amylase activity from Rhizopus preparations is difficult but relatively easy for Aspergillus preparations. Phosphatases, in minute amounts, may contaminate purified preparations, but the effect on the hydrolysis of starch may not be serious in the absence of α-amylase, the former enzyme being almost inactive for polysaccharide phosphates [47]. Two or three isoforms have been found in several enzymes of fungal origin, and the isoform with the highest molecular weight exhibits high activity not only for raw-starch granules but also for soluble polysaccharides such as glycogen because it has a starch-binding domain [48–50]. The enzyme is used for the determination of starch because it hydrolyzes starch specifically [51], and commercial preparations from Rhizopus contain weak α-amylase and completely hydrolyze starch [46].

9.2.1.5 Cyclodextrin Glucanotransferase

Cyclomaltodextrin glucanotransferase (EC2.4.1.19; 1,4-α-D-glucan 4-α-D-[1,4-α-D-glucano]-transferase [cyclizing], CGTase) is produced by several bacteria. The enzyme produces cyclomaltodextrins (cyclodextrins) from starch by intramolecular transglycosylation, a reaction referred to as cyclization. It produces branched cyclodextrins that link maltooligosaccharide chains to the ring with (1→6)-α-linkages from the branched portion of starch. The enzymes of Bacillus macerans, Klebsiella pneumoniae, and B. stearothermophilus preferentially produce α-cyclodextrin; those of B. megaterium, B. circulans, B. ohbensis, and alkalophilic Bacillus, mainly β-cyclodextrin; and those of Bacillus sp. AL6 and B. subtilis No. 313, γ-cyclodextrin [52].

CGTase also catalyzes the transfer of glucosyl residue of starch to the C-4 position of α-glucosyl-glucose, β-glucosyl-xylose, β-glucosyl-deoxy-D-glucosyl-glucose, 2-deoxy-β-glucosyl-glucose, and 3-O-methyl-β-glucosyl-glucose by intermolecular transglycosylation and thus produces various kinds of oligosaccharides. A series of maltooligosaccharides having 14C-glucose at their reducing ends was produced from cyclodextrins or starch and 14C-glucose [53]. This reaction is alternatively referred to as a coupling reaction. In addition, the enzyme catalyzes the hydrolysis of starch to maltooligosaccharides. These reactions are:

- Intramolecular reaction: Starch → α-CD, β-CD, and γ-CD (cyclization)
- Intermolecular reactions: CDs + 14G → G + 14G, G + 14G, G + 14G, ...
  (coupling reaction); G + G → G + G + G (disproportionating reaction)
- Hydrolysis: starch → G, G, G, ...
9.2.1.6 Phosphorylase

This enzyme is distributed widely in animals and plants with rabbit muscle and potato tubers as good sources. Starch and glycogen are degraded by phosphorolysis in the presence of inorganic phosphate (Pi) with starch phosphorolysis or glycogen phosphorylase $a$ and $b$ (EC2.4.1.1; 1,4-$\alpha$-D-glucan: orthophosphate $\alpha$-D-glucosyltransferase) from their nonreducing terminal residues into glucose 1-phosphate until four glucosyl residues remain on A chains [54] and three residues on the external B chains [25]. The reaction is fully reversible (Equation 9.3). It requires primer for the synthesis of glucan; the smallest effective molecule is G$_4$ and the least effective is G$_3$, but one form of the potato phosphorylase appears to synthesize glucan de novo [55]. Accumulated evidence suggests that the physiological role of phosphorylase is degradation, not synthesis. Previously, Walker and Whelan [54] had suggested that the outer chain length of the B chain of phosphorylase limit dextrin (φ-LD, Figure 9.4) was four, like that for the A chain, but this has been corrected to three by Bertoft [25]. Cori and Larner [56] suggested that two maltoses were produced from the outer B chain of φ-LD by $\beta$-amylase. This is consistent with the structure of Bertoft. Glycogen phosphorylase $b$ requires 5′-adenosine monophosphate as an activator, but muscle phosphorylase $a$ and starch phosphorylase do not require this cofactor. The limit of degradation of waxy maize amylopectin is approximately 47% [25]:

$$G_n + Pi \rightarrow \text{Glucose 1-phosphate} + G_{n-1} \quad (9.3)$$

9.2.2 Debranching Enzymes

The enzymes that hydrolyze (1→6)$\alpha$-D-glucosidic linkages in starch and glycogen are referred to as debranching enzymes. They increase iodine affinity (IA) and blue value (BV) and shift the $\lambda_{max}$ toward the longer wavelength of amylopectin and glycogen. Based on their actions, these enzymes may be classified into two types: direct and indirect debranching [57]. Isoamylase and pullulanase belong to the former, which hydrolyzes (1→6)$\alpha$-branch linkages directly or by one step. Amylo-1,6-glucosidase belongs to the latter, which
hydrolyzes only a single glucosyl side chain residue after one or repeated transfers of the oligosaccharide residues of side chains to other chains, as shown in Figure 9.4.

9.2.2.1 Isoamylase

Isoamylase was first found in yeast [58] and then in *Pseudomonas* [59], *Flavobacterium* (includes *Cytophaga*) [60,61], *Escherichia coli* K12 [62], and other sources [63,64]. Isoamylase completely degrades the branch linkages of amylopectin and glycogen endwise [65] to linear chains, only partially degrades (30–95%) the linkages of amylose [66–68], and degrades practically none of the pullulan linkages [65,69–72]. Therefore, it enhances the level of hydrolysis of amylose, amylopectin, and glycogen with β-amylase and increases iodine staining [60,69,72]. The substrate specificities have been investigated on a wide variety of oligosaccharides and polysaccharides by Kainuma et al. for *Pseudomonas* [71] and by Evans et al. for *Flavobacterium* [72]. Isoamylase hydrolyzes branched oligosaccharides at a much slower rate than amylopectin and glycogen [71,72]. It cannot release side chains of single glucosyl residues, can barely release maltosyl side chains, and exhibits strong action on maltotriosyl or longer side chains [71]. These specificities of action were confirmed on the branched cyclodextrins, which linked side chains of maltose, maltotriose, and higher homologs with (1→6)-α-D-linkages [61]. The enzyme hydrolyzes the β-limit dextrins of amylopectin and glycogen into mainly linear chains, maltosyl chains, and maltotriose and a small amount of maltose. It degrades wheat starch granules and produces a small amount (0.24%) of debranched products of amylopectin, but the action is very weak [73]. The *E. coli* enzyme [62] completely hydrolyzes amylopectin, but with difficulty, and it does not act on glycogen and pullulan. Phosphorylase limit dextrins of amylopectin and glycogen are the best substrate. The enzyme can produce branched cyclodextrins and branched oligosaccharides from altooligosaccharides and cyclodextrins by a reverse condensation reaction [74,75]. One should pay attention to this reverse reaction, but this action seems to be weaker than that resulting from pullulanase. Crystalline *Pseudomonas* isoamylases are commercially available and widely used for the structural analysis of amylopectin and glycogen and for the industrial production of G2 and other oligosaccharides. The kinetic properties of *Pseudomonas* and *Flavobacterium* isoamylases and *Klebsiella* pullulanase are compared briefly in Table 9.4. Evidently, isoamylases have superior activity for debranching amylopectin and glycogen to pullulanase.

9.2.2.2 Pullulanase

Pullulanase is produced by *Aerobacter aerogenes* (old name *Klebsiella pneumonieae*) [76], *Streptococcus mitis* [77], and other organisms [78–82]. It easily
hydrolyzes the (1→6)-α-D-glucosidic linkage of pullulan endwise, with the production of maltotriose oligomers and finally maltotriose and a trace amount of maltotetraose [71]; hydrolyzes amylopectin slowly but completely by exo-
wise action; and appears to produce A chains at the initial stage [70,83]. It
appears to partially hydrolyze glycogen very slowly (Table 9.4) and seems to
liberate predominantly A chains [65]. The branch linkage of amylose was also
hydrolyzed partially but completely with the cooperative action of
β-amylase [66]. 6→α-Maltosylmaltose was the smallest substrate [84], and pullulanase
cleaved (1→6)-α-interchain linkages between chains containing a minimum
of two glucosyl residues [85] but not a single glucosyl side chain, as with
isoamylase. The enzyme was competitively inhibited by cyclodextrins, espe-
cially cyclomaltoheptaose (β-cyclodextrin) [86]. An alkalophilic Bacillus No.
201-1 produces pullulanase, which hydrolyzes all the branch linkages of oyster
glycogen as well as waxy rice amylpectin [79], but another alkaline pullula-
nase [81] has little effect on amylose, amylpectin, and glycogen. Pullulanase
alone was unable to hydrolyze raw starch granules but synergistically enhanced
digestion by β-amylases [87].

9.2.2.3 Limit Dextrinase and R-Enzyme

A debranching enzyme called R-enzyme or limit dextrinase has been found
in a wide variety of higher plants, such as potato, broad bean, pea, rice, and
germinated barley, sorghum, and sweet corn [88–97]. R-enzyme and limit
dextrinase were discovered independently but were later recognized as the

---

**TABLE 9.4**

Kinetic Parameters of Isoamylase and Pullulanase

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Isoamylase</th>
<th>Pullulanase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Km × 10^4 (g/ml)</td>
<td>Vmax (U/mg) (%)</td>
</tr>
<tr>
<td>Flavobacterium</td>
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<td></td>
</tr>
<tr>
<td>Potato</td>
<td>2.0</td>
<td>411 (100)</td>
</tr>
<tr>
<td>Waxy maize</td>
<td>1.8</td>
<td>406 (99)</td>
</tr>
<tr>
<td>Glycogen</td>
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<td></td>
</tr>
<tr>
<td>Oyster</td>
<td>0.7</td>
<td>408 (99)</td>
</tr>
<tr>
<td>Rabbit liver</td>
<td>0.6</td>
<td>412 (100)</td>
</tr>
<tr>
<td>Pullulan</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

same enzyme [6]. The enzyme hydrolyzes α-limit dextrins, amylopectin, pullulan, and β-limit dextrins of amylopectin and glycogen, but not glycogen, which is partially hydrolyzed with bacterial pullulanase. The α-limit dextrins are rapidly hydrolyzed at a greater rate than pullulan. Oligosaccharides having a single glucosyl stub, such as panose, 6\(^2\)- and 6\(^3\)-α-D-glucosyl maltotriose [93], and α-D-glucosyl cyclomaltohexose, are not hydrolyzed [89]. The enzyme hydrolyzes greater than G\(_2\) chains linked to the G\(_2\) chain but favorably linked to G\(_3\) or G\(_4\). 6\(^4\)-α-Maltotriosylmaltotraose was the best substrate among the singly branched oligosaccharides for oat [90], rice [90], malted sorghum [89], broad beans [91], and pea [92]. Sweet corn and germinated barley enzymes were inhibited by α-cyclodextrin and especially β-cyclodextrin, as with pullulanase [86,93]. The germinated barley enzyme hydrolyzed isopanose into glucose and maltose [93]. The action of limit dextrinase depended on the concentration of substrate, and dilute solutions of amylopectin were not hydrolyzed [96]. The activity of barley enzyme increased considerably during germination, similarly to α-amylase [98]. The physiological role of the enzyme appears to be involved in the digestion of α-limit dextrins; hence, the term “limit dextrinase” is used rather than “R-enzyme” [88,93].

9.2.2.4 Amylo-1,6-Glucosidase

4-α-D-Glucanotransferase

This enzyme has been found in rabbit muscle and is a requisite for the complete hydrolysis of glycogen with phosphorylase [56]. Amylopectin and glycogen are hydrolyzed into glucose 1-phosphate and glucose in the presence of inorganic phosphate. Glucose 1-phosphate is produced from the (1→4)-α-linked glucose and glucose from the (1→6)-α-linked residue; therefore, branch linkages can be determined quantitatively from the amount of glucose liberated. The enzyme was discovered simply to be amylo-1,6-glucosidase (EC2.4.1.25; dextrin 6-α-glucosidase), because it produces only glucose from the φ-LD of glycogen; however, it was found later that the enzyme has the additional activity of 4-α-D-glucanotransferase (1,4-α-D-glucan: 1,4-α-D-glucan 4-α-D-glycosyltransferase; EC2.4.1.25), which catalyzes the transfer of α-1,4-glucan oligosaccharide chains from one chain to another in the connection of the structure of phosphorylase limit dextrin [54]. The enzyme first transfers a G\(_3\) or G\(_2\) residue from a side chain to a nonreducing residue of another chain and then liberates the resulting branched glucosyl residue shown in Figure 9.4. The enzyme has also been found in yeast [92,100]. Muscle enzyme preferentially transfers G\(_3\) rather than G\(_2\) from the substrate [100]. Yeast enzyme preferentially transfers G\(_2\) from G\(_4\), and G\(_3\) from G\(_5\) and G\(_6\). G\(_3\) is an acceptor but not a donor of the yeast enzyme [101]. 6-O-α-D-glucosyl-β-CD and 6-O-α-D-glucosyl-γ-CD are much better substrates than φ-LD for yeast and rabbit muscle enzymes, respectively [102].
9.3 SEPARATION AND DETERMINATION OF STARCH COMPONENTS

9.3.1 SEPARATION OF AMYLOSE AND AMYLOPECTIN

Several methods have been used for the separation of starch components [3,103,104], but none of them is entirely satisfactory from a qualitative and quantitative standpoint. Two main methods are currently used: (1) aqueous leaching, and (2) dispersion and precipitation. The first method was originally used by Meyer and his coworkers when they discovered amylose. Starch granules are leached with a large amount of water slightly above the gelatinization temperature and up to 80°C or more. Amylose has been preferentially leached out at lower temperatures, and the purity of the extracted amylose decreases at higher temperature because the amyllopectin of lower molecular weight has been extracted [105]. Pure linear amylose can be obtained at low leaching temperatures [104,105], but extraction is far from complete and large amylose molecules remain in the granules even when leached at higher temperatures. The amylose fraction extracted from wheat at 60°C [106] and 85°C [107] followed by crystallization with 1-butanol was found to contain one and three branches, respectively. To prepare pure amylose, an additional step of selective precipitation appears to be required.

Another popular method makes it possible to separate the two components nearly quantitatively, but usually 10% or more of the starch is lost during the process. The starch granules are completely dispersed in hot water or aqueous methyl sulfoxide, and amylose is precipitated as a crystalline complex by the addition of hydrophilic organic substances and cooling. Amylopectin is recovered from the supernatant by lyophilization or precipitation with alcohol, and amylose is purified by repeated recrystallization from 1-butanol-saturated water. The mixture of isoamyl alcohol and 1-butanol [3] or thymol [108,109] has been used for the first precipitation of amylose because these materials are efficient for the precipitation of amylose, leaving pure amyllopectin in the supernatant, but the mixture may precipitate some amyllopectin with long chains or intermediate fractions. The complete removal of lipids and disorganization of starches by dissolving in methyl sulfoxide, once or twice, and precipitation with acetone or ethanol has been an efficient method for fractionation [67,110]. Ultracentrifugation is necessary during the recrystallization to remove microgel-like amyllopectin from amylose [67]. The purity of amylose has been estimated by IA, but the method is not sensitive enough and is unable to detect the contamination of several percent of amyllopectin. Banks and Greenwood [111] have proposed an enzymic method of using commercial β-amylase that contains Z-enzyme (weak α-amylase). This is based on the fact that the enzyme can hydrolyze the amylose completely, leaving amyllopectin as its β-limit dextrin. The contaminating amyllopectin (Ap, %) can be estimated from Equation 9.4 [111]:

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Autoclaving for complete dispersion of starch is no longer done because it causes appreciable degradation, and the process is carried out under a nitrogen atmosphere below 100°C [67]. Size-exclusion chromatography (SEC) [112] has been used for the detection of contaminant amylopectin in amylose because amylopectin elutes before amylose. Monitoring of the eluent by a low-angle, laser-light-scattering photometer indicates clearly whether the eluent contains materials of different kinds [67].

**9.3.2 Determination of the Proportion of Amylose and Amylopectin**

Amylose and amylopectin are quantitatively determined by several methods, and variable results have been reported. The iodine reaction has been most commonly used for this purpose because it is specific, sensitive, and easy to determine qualitatively and quantitatively by a spectrophotometer. Blue value (absorbance at 680 nm under specified conditions) [113] has been used for the determination of the amylose content. Amylose gives high values (1.1 to 1.5) because it has a long linear portion, while amylopectin with many short linear parts gives very low values (0 to 0.16). Accordingly, the amylose content of starch is possible to assay from the BV of the starch, amylose, and amylopectin.

The iodine reaction with starch is also determined quantitatively by potentiometric [114] or amperometric titration [115,116]. Amylose molecules have a helical conformation and accommodate polyiodide ions, mainly I$_5^-$ [117], in the central tunnel of the helix [118]. During titration of the amylose with iodine solution, the electric current does not increase until all the amylose molecules are saturated with iodine, but amylopectin cannot easily make such a helical complex because it has short chains and has many branch linkages that interfere with or provide obstacles to the formation of a stable helical structure. The pure amylose prepared by several recrystallizations adsorbs 19.5 to 20.5 g iodine per 100 g, and amylopectin binds 0 to 1.2 g iodine per 100 g. The amylopectin fraction of amylomaize and similar so-called high-amylose starches binds considerably larger amounts of iodine. These values are referred to as the iodine-binding capacity or iodine affinity (IA). The amylose content of starch can be determined by BV or IA of starch, amylose, and amylopectin, from Equation 9.5:

\[
\text{Amylose content (\%)} = \frac{\text{BV or IA(starch) - amylopectin)}}{\text{BV or IA(amylose - amylopectin)}} \times 100
\]

Equation 9.5 is often conveniently simplified to Equation 9.6:

\[
\text{Apparent amylose content (\%)} = \frac{\text{IA of starch}}{20} \times 100
\]
in which the IA of amylose is supposed to be 20 (or occasionally 18 to 19) and that of amyllopectin 0; the value is referred to as the apparent amylose content. Occasionally, however, considerable errors have been found, because the iodine affinities of amyllopectins vary by specimen. For example, amyllopectins from some indica rice starches showed high IA and gave considerable higher apparent amylose contents than the real amylose content based on Equation 9.5 [116]. Table 9.5 shows the apparent and real amylose content of starches from various sources based on IA. Slightly different values are

<table>
<thead>
<tr>
<th>Starch</th>
<th>Iodine Affinity (IA)</th>
<th>Amylose Content (%)</th>
<th>Ref.</th>
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<tbody>
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</tr>
<tr>
<td>Koshihikari</td>
<td>3.69</td>
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<td>16.5</td>
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<tr>
<td>Sasanishiki</td>
<td>4.00</td>
<td>20.0</td>
<td>17.5</td>
</tr>
<tr>
<td>Rice, Indica</td>
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<td></td>
<td></td>
</tr>
<tr>
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<td>21.8</td>
<td>17.0</td>
</tr>
<tr>
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<td>5.08</td>
<td>25.4</td>
<td>18.5</td>
</tr>
<tr>
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</tr>
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<td>22.6</td>
</tr>
<tr>
<td>Barley, Bomi</td>
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<td>27.5</td>
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<td>Maize, normal</td>
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<td>21.5</td>
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<td>Water chestnut</td>
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<td>24.7</td>
<td>23.3</td>
</tr>
<tr>
<td>Chestnut</td>
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<td>19.6</td>
</tr>
<tr>
<td>Sago</td>
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</tr>
<tr>
<td>Low viscosity</td>
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<td>25.8</td>
<td>24.3</td>
</tr>
<tr>
<td>High viscosity</td>
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<td>24.9</td>
<td>24.4</td>
</tr>
<tr>
<td>Lotus</td>
<td>3.37</td>
<td>16.9</td>
<td>15.9</td>
</tr>
<tr>
<td>Kuzu</td>
<td>4.06</td>
<td>20.3</td>
<td>21.0</td>
</tr>
<tr>
<td>Sweet potato, Koganesengan</td>
<td>4.18</td>
<td>20.7</td>
<td>18.9</td>
</tr>
<tr>
<td>Yam, Nagaimo</td>
<td>4.29</td>
<td>21.5</td>
<td>22.0</td>
</tr>
<tr>
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<td>3.41</td>
<td>17.1</td>
<td>16.7</td>
</tr>
<tr>
<td>Arrowhead</td>
<td>5.20</td>
<td>26.0</td>
<td>25.6</td>
</tr>
<tr>
<td>Bracken, <em>Pteris aquilinum</em> L.</td>
<td>3.92</td>
<td>19.6</td>
<td>19.7</td>
</tr>
<tr>
<td>Lily</td>
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</tr>
<tr>
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<td>24.0</td>
<td>22.2</td>
</tr>
<tr>
<td>Potato</td>
<td>4.44</td>
<td>22.2</td>
<td>21.0</td>
</tr>
</tbody>
</table>
obtained when based on BV, as the chain-length dependencies of BV and IA are not the same. Alternatively, several methods based on different theories have been used that give considerably different values. In addition, pretreatment of the specimen also affects the results, as described below. Therefore, one should specify the method used to determine amylose content.

Complex formation with iodine is competitively inhibited by lipids, which make essentially the same helical complex with the linear portion of the glucan \[119\]. Starch often contains small amounts of lipids. Potato starch includes few or no lipids, and other starches from underground plants contain fewer lipids than those from cereals. Cereal starches may contain 0.6 to 1.3% lipids \[120,121\], resulting in considerable reduction of the IA, BV, and thus amylose content; therefore, it is necessary to remove the lipids completely by proper methods. The starch lipids included in the starch granule are not easily extractable without destruction of the granule. Table 9.6 shows the effect of extraction on the iodine affinity and the amylose content. Repeated extraction with hot 85% methanol \[122,123\] is far from complete and gives a lower value. Extraction with water-saturated 1-butanol \[120\] and 75% 1-propanol or 2-propanol-water \[120,124,125\] appears to be better but is still not complete. A satisfactory method is to gelatinize with dimethyl sulfoxide solution and precipitate twice with ethanol \[67\]. Morrison and Laignelet \[126\] defined apparent and total amylose as the values measured on native and lipid-free starches, respectively. The difference between total and apparent values implies the relative content of starch lipids. These authors have proposed that Equation 9.7 can be used to calculate amylose content from the BVs for cereal starches having amylose contents below 32%:

\[
\text{Amylose} \text{ (%)} = (28.414 \times \text{BV}) - 6.218
\] (9.7)
Equation 9.8 was proposed by Tester and Morrison [127] for barley starches. Although the computation appears simple, the experiments should be done under strictly controlled conditions:

\[
\text{Amylose} \, (\%) = (28.00 \times \text{BV}) - 4.65 \quad (9.8)
\]

Amylose and amylopectin are determined by SEC after debranching with isoamylase or pullulanase [128,129]. Debranched starch analyzed by this method using Sephadex G-75 [129], Sepharose CL-6B [128], or tandem columns of Toyo-Pearl HW55F and HW50SF gave three fractions in the elution order I, II, and III, as shown in Figure 9.5. The largest molecular fraction (FI) was identified as amylose based on the λ\text{max} and BV; FII and FIII originated from amylopectin, as judged by a distribution similar to that of amylopectin. Thus, the amounts of amylose and amylopectin can be determined from the amounts of these fractions. Fuwa and his coworkers [130–133] fractionated starch into four fractions with an additional intermediate fraction, which was located between FI and II (Figure 9.5). These fractions were defined based on the λ\text{max} of iodine coloration [130]. Fraction I, which was considered to be amylose, gave a λ\text{max} higher than 620 nm. The intermediate fraction was defined by a λ\text{max} in the range of 620 to 600 nm. Fractions II and III, which appeared to originate from amylopectin, had λ\text{max} values of 525 to 600 nm and below 525 nm, respectively. Because the intermediate fraction was supposed to be a mixture of amylose and amylopectin fractions [133] and did not appear as an independent peak, it seems reasonable to divide the chain-length distribution chromatogram into three fractions: I (amylose), II (long B chain), and III (short B and A chains). In general, amylose has several percent of short side chains similar to those of amylopectin [68,134], and some amylopectins have long chains such as those of amylose [116,134,135]; hence, the chromatographic method does not provide accurate values for the amounts of

![FIGURE 9.5 Fractions of debranched starch or amylopectin by SEC. Intermediate fraction by Fuwa is shown by dotted line.](image-url)
amylose and amylopectin. This method, however, is convenient and does give acceptable values for normal starches, except for some apparently high-amylose starches, which contain appreciable amounts of short-chain amylose and the extra-long-chain component of amylopectin [116].

The amylose is separable and determined directly by SEC on Sepharose CL-2B [136] or tandem columns of E-Linear and an E-1000 µ-Bondagel [137], because the amylopectin molecule has a several-fold greater molecular mass than amylose. By this method, slightly higher amylose contents (0.7 to 2.1%) of the five taro starches [138] and normal maize [133] than those obtained by the potentiometric method were reported. One of the difficulties with the SEC method is that amylopectin mainly elutes at the void volume but a small amount tails into amylase fractions, probably because of its high viscosity or the presence of small molecules, and it is difficult to separate by a baseline.

Amylose makes a helical complex with lipids by an exothermic reaction when it is heated and subsequently cooled in the presence of water. The complex is melted endothermically by reheating near or slightly above 100°C. Kugimiya and Donovan [139] demonstrated that the amylose content could be determined by measuring the melting enthalpy of the amylose–lysolecithin complex after heating and cooling starch in the presence of an excess amount of lipid and water. The initial heating and cooling were necessary for complete formation of the complex. They suggested –5.87 ± 0.1 cal/g of amylose for the standard enthalpy change of the complex. By this method the amylose content seems to be comparable to that measured by IA for potato (24%), tapioca (19%), lima bean (34%), wrinkled pea (65%), and waxy maize (0.6%), but slightly higher values are obtained for maize (33%) and wheat (37%). It has been suggested that the higher values are due to the presence of intermediate material; however, as described later, these amylopectins link the extra-long chains as revealed by chain-length distributions [140,141] and relatively high blue values, and this is probably the reason for the higher amylose content. The presence of starch lipid does not affect the results, so defatting is unnecessary. This method is simple and, in addition, gives important information on the gelatinization of the starch; however, it is often used as a standard method.

Concanavalin A precipitates exclusively amylopectin from an aqueous solution by binding at nonreducing terminal residues [142,143]; therefore, amylose can be determined by the total carbohydrate remnant in the supernatant. This method has been utilized for both analytical and preparative purposes and has given values of amylose content comparable to those obtained by iodine titration methods on potato, waxy rice, and rice starches but considerably lower values on wrinkled-pea starch [142] and amylomaize [144]. The lower values appear to be due to the presence of intermediate materials.
9.4 SOME METHODS FOR STRUCTURAL ANALYSIS

9.4.1 AVERAGE MOLECULAR WEIGHTS

Polysaccharides are mixtures of molecules with varied sizes and shapes, and their molecular weight is characterized by some average values obtained by different methods, such as end-group assay, osmotic pressure, viscosity and light-scattering measurements, and ultracentrifugation analysis. These molecular weights are defined as follows on a mixture comprised of \( N_i \) molecules with molecular weight \( M_i \).

The number-average molecular weight (\( \bar{M}_n \)), defined in Equation 9.9, is obtained by end-group assays or osmotic measurement:

\[
\bar{M}_n = \frac{n_1M_1 + n_2M_2 + \dots + n_nM_n}{n_1 + n_2 + \dots + n_n} = \frac{\sum_{i=1}^{n} n_i M_i}{\sum_{i=1}^{n} N_i} \quad (9.9)
\]

The weight-average molecular weight (\( \bar{M}_w \)) is defined in Equation 9.10 and is determined by light-scattering measurement:

\[
\bar{M}_w = \frac{n_1M_1^2 + n_2M_2^2 + \dots + n_nM_n^2}{n_1M_1 + n_2M_2 + \dots + n_nM_n} = \frac{\sum_{i=1}^{n} n_i M_i^2}{\sum_{i=1}^{n} n_i M_i} \quad (9.10)
\]

The \( z \)-average molecular weight (\( \bar{M}_z \)) shown in Equation 9.11 is determined by ultracentrifugation:

\[
\bar{M}_z = \frac{n_1M_1^3 + n_2M_2^3 + \dots + n_nM_n^3}{n_1M_1^2 + n_2M_2^2 + \dots + n_nM_n^2} = \frac{\sum_{i=1}^{n} n_i M_i^3}{\sum_{i=1}^{n} n_i M_i^2} \quad (9.11)
\]

The viscosity-average molecular weight (\( \bar{M}_v \)) is defined as follows:
where \( a \) is the exponent of the Mark–Houwink–Sakurada equation:

\[
[\eta] = KM^a \tag{9.13}
\]

The \( K \) and \( a \) values are specific for each material and dependent on solvent and temperature. Many \( K \) and \( a \) values have been proposed for amylose and its derivatives from different laboratories [103,145]. Amylose is a mixture of polymers with varied branching; therefore, theoretically, it gives specific \( K \) and \( a \) values for each specimen. This is also true for amylopectins with varied frequencies of branching. In fact, \([\eta]\) (ml/g, in 1-M NaOH at 22.5°C) has been shown to have a much better correlation with molar fractions of branched amylose than with molecular weight in seven rice amyloses because they had similar molecular weights [135]. However, good correlations were found between the \([\eta]\) and weight-average degree of polymerization (\(\overline{DP}_w\)) (Equation 9.14) and number-average degree of polymerization (\(\overline{DP}_n\)) (Equation 9.15) in amyloses from wide sources (19 specimens) [135] because the amounts of branched molecules are relatively constant and usually in the range of 30 to 40%:

\[
[\eta]_{\text{amylose}} = 0.41 \cdot (\overline{DP}_w)^{0.78} \ (r = 0.96, \ n = 19) \tag{9.14}
\]

\[
[\eta]_{\text{amylose}} = 0.51 \cdot (\overline{DP}_n)^{0.53} \ (r = 0.88, \ n = 19) \tag{9.15}
\]

If the material is homogeneous, such as enzymes, those values are identical, but when the material is a mixture of various sizes these values are in the following order:

\[
\overline{M}_n < \overline{M}_v < \overline{M}_w < \overline{M}_z
\]

9.4.2 Determination of Reducing Residue and \( \overline{M}_n \)

\( \overline{M}_n \) is easily determined by colorimetric methods for reducing residues. Occasionally, methods that have been devised for the determination of monosaccharides have been applied to oligosaccharides and polysaccharides, perhaps out of carelessness. For example, colorimetric assays for glucose by the Somogyi [146] method using the colorimetric reagent of Nelson [147] and the Park–Johnson method [148], based on the formation of Prussian blue, have been used widely, but they are not suitable for mixtures of oligosaccharides
and polysaccharides because they give decreased and increased reducing power per mole with increased molecular size, respectively. These methods have therefore been modified to give the same reducing value for a series of maltooligosaccharides. Such modifications include extending the heating period to 30 minutes in the Somogyi method [149], reducing the pH of the reaction mixture using carbonate–bicarbonate instead of carbonate, and omitting the surfactant that causes precipitation of amylose [66]. The modified Park–Johnson method is sensitive, and it is possible to determine molecular weights up to about 20,000 DP.

The reducing end can be determined enzymatically after reduction with borohydride. The reduced terminus and other glucosyl residues give sorbitol and glucose, respectively, by acid hydrolysis. Sorbitol is converted into fructose and NADH with sorbitol dehydrogenase (EC1.1.1.14) in the presence of NAD*. The NADH is determined photometrically at 340 nm or possibly fluorimetrically with higher sensitivity at excitation 340 nm and emission 456 nm [150]. The glucose produced is determined by the Somogyi–Nelson or other suitable methods. The DP is calculated from ($\mu$mol of glucose)/($\mu$mol of sorbitol). This method is suitable for routine use, but it is tedious to remove borate that interferes with the action of the enzyme.

Alternatively, the reducing residue is determined by labeling with $^3$H–NaB$_3$H$_4$ [151], a method that was developed by Richards and Whelan [152]; however, because of the high control value caused by radioactive contaminants in NaB$_3$H$_4$, it is not suitable for high polymers such as amylose. This method was improved by the use of glass fiber filters instead of filter paper for washing the residual reagent, and the radioactive contaminants may be removed from the polysaccharide product by gel filtration. This method is useful for determination of the reducing residue.

For debranched amylpectin or short-chain amylose (up to DP 80), the M$_n$ of each fraction from size-exclusion chromatography can be estimated using the BV and $\lambda$ max under specified conditions (concentrations of I$_2$ and KI, pH, and temperature) because amylose gives increased blue values and $\lambda$ max values with increases in the DP [153]. Banks et al. [154] and Fales [155] proposed Equation 9.16 and Equation 9.17, respectively, for determination of the number-average molecular weight. These methods are applicable to specimens having a narrow molecular weight distribution; otherwise, a considerable error may be produced. Equation 9.17 is applicable for CL between 35 and 100 [156]:

\[
\text{DP}_n = \frac{1.025 \cdot \lambda_{\text{max}} (\text{nm})}{100 - 0.1558 \cdot \lambda_{\text{max}} (\text{nm})}
\] (9.16)

\[
\text{DP}_n = \frac{3290}{635 - \lambda_{\text{max}} (\text{nm})}
\] (9.17)
9.4.3 Determinations of Nonreducing Terminal Residues and CL

The frequency of branching in amylopectin and amylose is quantitatively determined by the number of branch points or nonreducing terminal residues. Methylation is the normal technique for the analysis of branch positions in sugar residues; however, because it is known that the branch occurs only at C-6 positions in starch, methylation has not been employed except in special cases because it is time consuming and requires a large amount of specimen and great skill. Simple periodate oxidation has also been used for determination of the nonreducing residue, but not frequently because of overoxidation, which produces an increased amount of formaldehyde and formic acid; moreover, the reaction is difficult to control.

Alternatively, periodate oxidation followed by reduction of oxidized polysaccharide and hydrolysis with acid (i.e., Smith degradation) has been used for the analysis of nonreducing terminal residues and branch linkages. This is because the overoxidation, which proceeds stepwise from a reducing residue, interferes very slightly with the production of glycerol from nonreducing residues; therefore, nonreducing residues are determined from the amount of glycerol produced [157].

Usually, periodate oxidation is carried out at lower than room temperature to minimize overoxidation, but the reaction is slow and takes several days to complete. Increasing the temperature to 50°C or even 100°C results in completion of the reaction in less than 1 hour or even 10 minutes without any effect of overoxidation. In addition, every step of the Smith degradation was shortened by Hizukuri and Osaki [158] to allow completion within several hours. Glycerol was measured by the coupled reactions of glycerol kinase and glycerol phosphate dehydrogenase photometrically at 340 nm or fluorometrically [112] at an excitation wavelength of 340 nm and at an emission wavelength of 456 nm based on the reduction of NAD⁺ to NADH. Photometric assay [158] is suitable for oligosaccharides and amylopectin, and fluorometric assay is used for amylose [66,112] because of its high sensitivity. CL is obtained by dividing the total carbohydrate, such as glucose, by the number of nonreducing residues:

\[
\text{CL} = \frac{\text{total carbohydrates, as glucose}}{\text{number of nonreducing terminals}}
\]  

(9.18)

The average number of branch linkages \(\overline{NB}\) is obtained by subtracting 1 from \(\overline{CL}\):

\[
\overline{NB} = \overline{CL} - 1
\]  

(9.19)
9.4.4 **Analysis for Molecular Weight Distribution**

9.4.4.1 **Size-Exclusion Chromatography**

Amyloses and amylopectins from various sources are mixtures of molecules with varied molecular weights in certain ranges. The most popular technique for analyzing molecular weight distribution is SEC, also known as gel permeation chromatography. Some precautions with this method include selecting a gel with the correct pore size, using a good solvent, and applying a minimum amount of specimen both by volume and by concentration. Often a single-pore column does not give good separations for specimens with widely varying molecular weights, so two or three columns with different pore sizes in tandem or mixtures of gels with different pore sizes have been employed. The order of elution, or retention time, is dependent not only on molecular size but also on viscosity. Empirically, it has been established that the retention time or elution volume is inversely proportional to \( \log [\eta] \cdot [M_w] \). The hydrodynamic volume, a product of limiting viscosity number \([\eta]\) and molecular weight (M), has the same function of elution volume; therefore, the plots of \( \log [\eta] \cdot [M_w] \) vs. retention time give a single straight line for various materials under the same conditions. This plot is referred to as the universal calibration line. The \([\eta]\) is dependent on the branching of the polymers, if the polymers are composed of the same monomers. Branched molecules are more compact and give smaller \([\eta]\) than linear molecules of the same molecular weight and therefore elute later. The molecules with fewer branches elute earlier. Figure 9.6 shows

![Figure 9.6](image_url)


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this relationship for amylose, amylopectin, and glycogen [159]. The molecular weight can be estimated from the retention time only when the same kind of polymer is used as a reference material.

Occasionally, different polymers are erroneously used as references; therefore, the best method is to monitor the molecular weight directly. A convenient method for this is to use low-angle laser-light-scattering photometer (LALLS) and differential refractometer (RI) detectors in tandem [160,161]. This technique provides weight-average molecular weights (M<sub>w</sub>), whether at specified elution points or as fractions based on the ratio of output of the two detectors, LALLS and RI, which are proportional to molecular weight and concentration, respectively. In addition, the number-average molecular weight (M<sub>n</sub>) is also obtained [159]. Precolumn derivatization of reducing residues with a fluorophore followed by SEC has been successfully applied for amylose [161a], amylopectin [161b], and debranched amylopectin [161c]. The high-performance liquid chromatography (HPLC) system for such analyses is equipped with a fluorescence detector and an RI. A linear relationship between DP<sub>n</sub> and the ratio of detector responses — RI to fluorescence (both measured as peak area) — was observed in the DP<sub>n</sub> ranges of 6 to 440 [161c] and 521 to 4400 [161a], indicating that the fluorescence intensity per labeled α-glucan molecule is independent of the DP (DP<sub>n</sub>) of the specimen. Therefore, DP<sub>n</sub> and DP at a given elution position can be determined simply from a ratio of the responses by the two detectors. This technique was applied to analysis of the CL distribution of the main chain of amylopectin [161c]. Amylopectin molecules were labeled and then debranched with isoamylase so the main chains of the molecules could be differentially detected by fluorescence and SEC of debranched amylopectin [161c].

### 9.4.4.2 Ion-Exchange Chromatography

High-performance anion-exchange chromatography using a pulsed-amperometric detector is a useful technique for the qualitative and quantitative analysis of monosaccharides, oligosaccharides, and homoglucan series because of its high resolution and sensitivity [162]. The debranched amylopectin is separated into components, each up to DP 50 to 60 (Figure 9.7) [163]. Because a series of (1→6)-α-D-glucans was found to elute faster than the corresponding (1→4)-α-D-glucans [162], the latter contained one (1→6)-α-linkage with DP<sub>n</sub> eluted between the positions of pure (1→4)-D-glucans with DP<sub>n</sub>−1 and DP<sub>n</sub>, thus linear and branched molecules were easily distinguishable [164]. The problem with this method is that the detector response is not constant for individual chains. According to Koizumi et al. [163], responses of the detector with regard to C–OH decrease with increasing molecular size, and this may be due to the increased steric hindrance. The relative response of pulsed amperometric detection up to DP 17 has been measured by Koizumi et al. [163]. Wong and Jane [164a] took a different approach to obtain quantitative results by pulsed...
amperometric detection. The enzyme (glucoamylase) reactor was inserted between an analytical column and a detector, so separated maltooligosaccharides are first converted to glucose and then introduced to the detector. Thus, the DP dependence of the detector response is avoided and a single calibration curve for glucose can be used for the quantification of peaks for different degrees of polymerization.

9.4.4.3 Electrophoresis

Labeling reducing terminal residues of carbohydrate molecules with fluorophores that have functional groups such as carboxyl and sulfonyl groups results in (1) charges under appropriate pH that cause migration of the derivatized carbohydrate molecule in an electric field, and (2) means proportional to the number of molecules. The maltooligosaccharides/α-glucan chains labeled in such a way can be separated by slab gel or capillary electrophoresis. Morell and colleagues examined the efficiency of labeling of maltooligosaccharides with 8-amino-1,3,6-pyrenetrisulfonic acid (APTS) and reported that the efficiency is practically constant in the DP range of 3 to 135; therefore, the method is suitable for analyzing debranched amylopectin [164b]. Separation by capillary electrophoresis [164b] is comparable to that obtained by high-pH, anion-exchange chromatography (HPAEC), and, without any correction of detector response, fluorescence profiles represent molar distributions of unit chains of amylopectin.

9.5 AMYLOPECTIN STRUCTURE

9.5.1 HISTORICAL SURVEY

The classic structures of the laminated and comb-like models for starch were proposed by Haworth [165] and Staudinger [166], respectively. These were based on analysis by methylation and osmotic pressure or viscosity measurements as early as 1937. After successful separation of amylose and amylopectin, Meyer et al. [1] analyzed the structure of the amylopectin by chemical, physical, and enzymatic methods and proposed the randomly branched, bush-like structure. Key information regarding the structure was obtained by enzymatic analysis. Meyer et al. degraded amylopectin with $\beta$-amylase and subsequently with $\alpha$-glucosidase, which hydrolyzed branch linkages exposed at the surface, and repeated this stepwise degradation. The bush-like structure was deducted from the decreasing yields of the products on repeated hydrolysis. At that time, the nature of the $\alpha$-glucosidase was not clear, but from present knowledge it seems to be $\alpha$-1,6-glucosidase. The first strong support for this structure was provided by Larner et al. [167]. They degraded amylpectin and glycogen with muscle phosphorylase and subsequently with amylo-1,6-glucosidase and found that two of three cycles of digestion were necessary for the complete degradation of amylopectin and glycogen. They also observed decreasing yields of the products as the cycles of degradation progressed. These results fitted into Meyer’s structure. Although Cori and Larner’s asymmetrical structure for phosphorylase limit dextrin (Figure 9.4) was later corrected by Walker and Whelan [54] and Bertoft [25], their work is of significance in understanding the structure of amylopectin.

In the 1960s, questions arose regarding Meyer’s irregular structure of amylopectin, particularly with regard to (1) how amylopectin molecules make crystalline starch granules, (2) why amylopectin molecules exhibit higher viscosities than glycogen of similar molecular weight, and (3) the reasons for the bimodal chain-length distribution in amylopectin. In answer to these questions, in 1970 Whelan et al. [168] submitted a revision of the structure proposed by Meyer, and Nikuni [169], French [170], and other investigators [83,171–173] proposed cluster structures. Figure 9.8 shows some of these structures. Several lines of evidence support the cluster structure, and it is widely accepted.

9.5.2 GENERAL PROPERTIES

Amylopectin is generally characterized by blue value, $\lambda_{\text{max}}$ of iodine coloration, iodine affinity, intrinsic viscosity, $\beta$-amylolysis limit, molecular weight, A-to-B-chain ratio, and chain-length distribution. The general analytical values obtained for amylopectins, fractionated in the authors’ laboratory by the dispersion method of Schoch [3], are compiled in Table 9.7. These values are specific by plant species and variety and are variable within certain ranges due
FIGURE 9.8 Structures of amylopectin proposed by various investigators. A, B, and C chains are defined in the text. The structures of Whelan, French, Nikuni, and Hizukuri are cited from references 168, 170, 169, and 172, respectively.
to their growth conditions and postharvest treatment. Usually, considerable variations have been reported for each plant species. These variations arise mainly from the different methods of analyses, personal errors, and historical background of the specimens.

Analytical data based on iodine coloration are sensitive to the presence of small amounts of higher-chain components. Specimens with wider distributions of chain length may give greater $\lambda_{\text{max}}$ values and larger BV and IA values compared to specimens having the same average chain length. A small amount of extra-long chains (ELCs) greatly increases these values, and the purity of the specimen should be confirmed by SEC [116,137] to determine whether or not they contain amyllose. In general, $\lambda_{\text{max}}$ is in the range of 530 to 550 nm, BV $< 0.2$, and IA $< 1.0$. Amylopectin fractions of amylomaizes [174] and wrinkled peas [136] gave exceptionally higher values and are generally classified as intermediate. The reported $M_w$ of amylopectins from various sources falls within the range $4-5 \times 10^8$ ($2.5-3 \times 10^6$ DP) [175]. The DPn of rice amylopectins ranged from 5000 to 13,000 [130] and that of sago amylopectins from 10,000 to 40,000 [176]. The range of $M_w$ distribution seems to be very wide. Amylopectin appears to be easily degraded by shear force in concentrated solutions [175]. The large $M_w$ by physical aggregation does not appear likely because light-scattering measurements in disaggregating solvents such as 8-M urea and 15% magnesium chloride gave the same molecular weight [175]. The $[\eta]$ is in the range of 140 to 180 (ml/mg) and is dependent on molecular shape, chain-length distribution, and the amount of the ionized groups, in addition to molecular weight. The rice amylopectins, which have abundant extra-long chains, gave large $[\eta]$ values [116]. $\beta$-Amylolysis limits are in the range of 55 to 61%.

### 9.5.3 COVALENTLY BOUND PHOSPHATES

Starch contains two types of phosphorus in small quantities: esterified phosphates and phospholipids. The latter type is can be removed by using proper solvents but the former remains; hence, they are easily distinguishable. The ester phosphate groups are found exclusively in amylopectin [177,178]. Potato amylopectin contains 200 to 1000 ppm of the ester phosphorus [179] and those from other roots contain 40 to 150 ppm; cereals contain less than 20 ppm [180], with the exception of amylomaize, which contains 110 to 260 ppm [174] (Table 9.7).

The structures and functions of the phosphate ester have been studied in detail in potato starch. Posternak found that the phosphate groups were located at C-6 primary hydroxyl groups [181,182]. Hizukuri and coworkers quantitatively determined the phosphate at C-6 and observed the presence of an unknown phosphate [149], later identified as glucose 3-phosphate [183], and they also noted that trace amounts of glucose 2-phosphate were present. The ester at C-3 [$P_3$] has also been found in sweet potato amylopectin [184].
<table>
<thead>
<tr>
<th>Source</th>
<th>Blue Value (BV)</th>
<th>Iodine Affinity (IA)</th>
<th>$\lambda_{max}$ (ppm)</th>
<th>P0 (ppm)</th>
<th>P6 (ppm)</th>
<th>$\beta$-Amylolysis Limit (%)</th>
<th>CL</th>
<th>$[\eta]$</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rice (japonica)</td>
<td></td>
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<td></td>
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<td>116</td>
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<td>0</td>
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<td>58</td>
<td>—</td>
<td>57</td>
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<td>0</td>
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<td>90</td>
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<td>555</td>
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<td>650</td>
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<td>23</td>
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These ester linkages, especially at C-6 [P6], are more stable than the (1→4)-α-glucosidic linkages against acid hydrolysis; consequently, glucose 6-phosphate and glucose 3-phosphate have been identified in the acid hydrolyzate [149,183]. Glucose 6-phosphate is specifically determined by an enzymatic method using glucose 6-phosphate dehydrogenase and NADP⁺, but no such specific method is available for glucose 3-phosphate. These phosphate esters were identified and determined by 31P nuclear magnetic resonance (NMR) [185–189]. The α,λ-phosphodextrin from potato showed signals at chemical shifts of 1.62 (P3) and 1.95 (P6) ppm, with sodium phosphate as the internal standard at pH 8.0 and 4.52 (P3) and 4.85 (P6) ppm with 95% phosphoric acid as the standard [187,188]. Phosphodiester linkages in lecithin gave a broad peak between −1.0 and −1.0 ppm (95% phosphoric acid as the standard), and the chemical shift of inorganic phosphate was 2.9 ppm [187]. Lim and Seib [186] identified internal P2, P3, and P6 and P6 nonreducing residues of the α- and α,λ-limit dextrins of potato starch and chemically phosphorylated amyllose and wheat starch by 31P-NMR. The 31P chemical shifts of P6 and P3 linked to the inner residues in α-limit dextrins shifted slightly toward downfield after removal of the outer residues by glucoamylase. Thus, phosphate monoesters at nonreducing and internal residues were distinguishable. Based on the 31P-NMR spectra, Lim et al. [188] confirmed that larger amounts of starch phosphates are on C-6 than on C-3 in potato, sweet potato, lotus, arrowroot, green pea, and mung bean, whereas lima bean contains almost the same amount. They quantitatively determined phosphorus, as monophosphate and phospholipid, on starches from various origins, as shown in Table 9.8.

Waxy varieties of amaranth, rice, and maize appeared to bind inorganic phosphate (10 to 50 ppm) tightly and could not be extracted with water. The reason for this phenomenon is unknown. 31P-NMR seems to be useful for the analysis of phosphorus in starch because various kinds of phosphate can be qualitatively and quantitatively analyzed together, but a higher sensitivity is desirable.

Autoclaving, as in heat-moisture treatment, liberated inorganic phosphate (Pi) mainly from P3 [149], and storage of starch for years was also found to increase Pi and consequently decrease organic phosphate (Po) [190] with an increased relative amount of P6. One reason for the variation in relative P6 content appears to be storage conditions, as inorganic phosphate was found to be liberated during storage at room temperature [190], thus increasing the ratio of P6 to P3. Potato grown at lower temperatures was found to contain more phosphorus [191]. In a study by Bay-Smidt et al. [189], the phosphorus content at C6 increased about 50% from the cortex toward pith, but conversely the starch content decreased. Jane and Shen [192] found, by stepwise chemical gelatinization, that phosphorus was located densely in the cores of potato starch granules together with amyllopectin. The CLn of phosphorylated chains of potato starch has been found to be −40, indicating that these phosphates
link mainly to long B chains. The detailed analysis suggested 60% and 30% in the outer and inner sections, respectively, of B chains and 10% in A chains [193]. It has been suggested that the incorporation of phosphate into starch occurs parallel to starch synthesis in potato slices [194]. The glucosidic linkages at the vicinity of these phosphate groups are immune to α-amylase, so several phosphorylated oligosaccharides are produced by hydrolysis with α-amylases [10]. Recently, a protein called R1, which was identified originally as a protein bound to the surface of starch granule, was reported to be an α-glucan, water dikinase that is responsible for starch phosphorylation [194a]. A perspective on future research in this field has been provided by Blennow et al. [194b].

---

TABLE 9.8
Phosphorus Contents of Starches

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<tr>
<th>Source</th>
<th>P₀ (ppm)ᵃ</th>
<th>P₆ (ppm)ᵇ</th>
<th>P₆/P₀ (%)ᵇ</th>
<th>Monoester-Pᵇ</th>
<th>Lipid-Pᵇ</th>
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<td>117–132</td>
<td>82–99</td>
<td>66–70</td>
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<td>129</td>
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<tr>
<td>Kuzu</td>
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<td>—</td>
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ᵃ Defatted with 80% dioxane for 48 hours.
ᵇ Measured by ³¹P-NMR [188].
9.5.4 Ratio of A Chain to B Chain

To characterize and analyze the structure of amylopectin, Peat and coworkers [195, 196] classified the chains of amylopectin as A, B, or C. The A chain is linear and links to C-6 of the other chains by its reducing residue, the B chain carries the A or other B chains at its C-6, and the C chain has a single chain having a reducing residue. The structures proposed by Haworth, Staudinger, and Meyer are characterized by the A:B chain ratio as 0, ∞, and 1, respectively. From the yields of G₂ and G₃ originating from the A chain by hydrolysis of the β-LD of waxy cornstarch with R-enzyme, Peat et al. [196] confirmed the structure proposed by Meyer. Since then, the A:B ratio has been taken as a key value for characterization of the structure of amylopectin. Marshall and Whelan [197] determined the A:B ratio from the reducing powers produced by the extent of hydrolysis of β-LD with isoamylase alone (I) and the combined action of isoamylase and pullulanase (II) using Equation 9.20:

\[
\frac{A}{B} = \frac{2[I - I]}{[2I - II]} \quad (9.20)
\]

This determination is based on the assumption that (1) G₂ and G₃ are produced only from A chains, (2) G₂ and G₃ are produced in equal amounts because they originate from A chains with even and odd chain lengths, (3) isoamylase debranches all the branch linkages of β-LD except the one linked to maltosyl stubs, and (4) the combined action of the two enzymes liberates all the branch linkages of β-LD. However, isoamylase slowly liberates a small amount of G₃, and this would cause considerable error. The A-to-B-chain ratio can be determined from the molar amounts of reducing sugars (RSs) and G₂ and G₃ produced from the β-LD by complete debranching with isoamylase and pullulanase or possibly by pullulanase alone using Equation 9.21:

\[
\frac{A}{B} = \frac{[G₂ + G₃]}{RS - [G₂ + G₃]} \quad (9.21)
\]

The RSs may be determined alternatively as nonreducing residues by Smith degradation; the total amounts (in moles) of G₂ and G₃ can be substituted by 2G₂ in Equation 9.22. The oligosaccharides G₂ and G₃ are determined after separation by SEC [198] or by HPLC using suitable columns. Because a small amount of G₃ is produced from the B chains in the β-LD of amylopectin and a large amount of G₂ from those of glycogen β-LD [164, 199], Equation 9.21 may be modified to produce Equation 9.22:

\[
\frac{A}{B} = \frac{2[G₂]}{RS - [2G₂]} \quad (9.22)
\]
The A:B ratio is also measured by the hydrolysis of φ,β-dextrine, in which all the A chains are theoretically maltosyl residues, with isoamylase alone (I) and with a joint action of isoamylase and pullulanase (II). The former liberates only B chains and the latter liberates all the A (G₂) and B chains. Therefore, II – I is equal to A chains. One should keep in mind that isoamylase could liberate a small amount of G₂ (A chains). In addition, some A chains of glycogen that are of maltosyl or maltotriosyl length [164] remain intact after phosphorysis and could cause errors. However, such short chains are present in negligible amounts in amylopectins.

As shown in Table 9.9, various ratios of A and B chains were found by different investigators [83,197–205]. This variation seems to be due to incomplete debranching [83] or partial debranching of G₂ stubs by isoamylase and, in particular, determination of the reducing sugars maltose and amylotriose. Where the A:B ratio is 1 and each assay of total reducing sugars G₂ and G₃ has a 5% error, the found value of A:B is in the range 0.75 to 1.22; thus, slight errors in the determination of these sugars result in considerable error in the A:B ratio. The most acceptable value for amylopectin appears to be 1.0–1.4:1, and obtaining accurate critical measurements is a subject for future investigation. Hizukuri [172] reported that the A:B ratio decreased with an increase in the chain length of amylopectin from the chain-length distribution of
debranched amylopectin, assuming the shortest chain fraction is the A chain. However, the values should be regarded as relative values because peaks in the chromatogram were overlapping.

9.5.5 Distribution of Chain Length

The CLs of most amylopectins are in the range of 18 to 24, and the chain-length distribution is characteristic for each plant species. The CL distribution is analyzed by SEC or HPAEC with pulsed amperometry after debranching of amylopectin with isoamylase. In early years, debranching was performed by pullulanase, which has a much weaker action than isoamylase and might cause incomplete debranching. Conventional SEC was carried out on one or two connected columns packed with Bio-Gel P-10 [205], or P-6 [206], Sephadex G-50 [207] or G-75 [208], Toyo-Pearl HW55–HW55SF [133], or similar support. The resulting chromatograms indicated bimodal or trimodal distribution with a large peak at a DP of about 10 to 20 (FIII), a medium peak at about 45 DP (FII), and a small peak with variable intensity at higher DP regions (FI) (Figure 9.5). Peak F1 may include incomplete debranched material.

When whole starch is debranched, amylose emerges at the FI position. Peak FIII appears to include A plus short B chains, and FII contains long B chains. No FI fraction has been found in waxy varieties of rice, maize, and barley [115,206], but small amounts have been found in amylopectins of various normal starches. These are denoted as extended or extra-long chains [116,135]. Hizukuri and his coworkers [135] investigated the CL distribution of rice amylopectins with varying IAs by high-performance SEC and found that higher IAs had increased amounts of ELCs. The rice amylopectins with IAs of 0.38 to 2.57 had an ELC fraction of 6 to 20%. A total of 10 to 11% of this fraction was found in normal maize [140]; 4 to 6% in wheat amylopectins [209]; 2.6% and 1.8%, respectively, in sago of low and high Bravendor viscosity [176]; 6 to 8% in sweet potato [184]; 3% in water chestnut [210]; 0.8% or less in potato [179]; and 4% and 2%, respectively, in amylo maize amylopectins Hylon and Hylon 7 [179]. Hylon 7 has a slightly higher amount of F2 (26%) than Hylon (21%). It appears to be a general trend that amylopectins with longer CLs have more F2 fraction, and the longer CL is not due to a shift of the distribution range [211] but to an increase of F2 and consequent decreases in the F2/F3 ratio. The ratio F2/F3 is often used to characterize the chain-length distribution of amylopectin [133,212,213]; for example, in maize mutant genotypes with an Oh43 inbred line, the F2/F3 ratios of normal maize, amylose extender (ae), brittle 1 (bt 1), brittle 2 (bt 2), horny (h), shrunken 2 (sh 2), sugary 1 (su 1), and waxy (wx) were determined to be 2.8, 1.0, 3.0, 2.4, 5.6, 2.8, 3.8, 4.6, and 2.6, respectively [213]. The ae gene decreased the ratio of their mutants [133,213] and the du1 (du1) gene increased the ratio.
These chain-length distribution profiles from single [133], double, and triple mutants of genotypes with an Ob43 maize inbred line background indicate that the structure of amylopectin is due to genetic factors [212,213].

By selecting column TSK G3000SW and two G2000Sw packed columns [172] in tandem, or other column combinations [211], Hizukuri [172] observed a polymodal distribution (Figure 9.9) with five peaks — A, B1, B2, B3, and B4. The ELC component was not observed in these chromatograms, because the ELC component had been thought to be amylose and was removed as precipitate with 1-butanol complex, except for the case of wheat. The FI fraction in Figure 9.5 seems to be a mixture of fractions EL and B4, FII to be B2 and B3, and FIII to be A and B1. The average chain lengths of the B1, B2, and B3 fractions are in the range of 20 to 24, 42 to 48, and 69 to 75, respectively, and the relative lengths are roughly 1:2:3. This implies that the A, B1, B2, B3, and B4 chains may be involved in the formation of one, two, three, and more than four clusters. This polymodal distribution supports only a cluster structure. The differences between B2 and B3 and between B3 and B4 may be a single cluster length, but these values do not agree, based on the assumption that all the B2 chains do not extend over the two clusters, whereas the B3 chains connect three clusters and go through the entire single cluster.
Therefore, B3–B2 rather than B2–B1, would be a cluster length, although the boundary between B3 and B4 is generally vague, making it difficult to estimate the DP of the B4 fraction with reasonable accuracy. This distribution indicates that 80 to 90% of the chains (A and B1) comprise a single cluster, approximately 10% of the chains (B2 chain) are involved in the connection of two clusters, 1 to 3% of the chains seem to connect three clusters, and only 0.1 to 0.6% of the chains may connect more than four clusters. The chain-length distributions of some amylopectins are summarized in Table 9.10. Potato and other B-type (crystalline structure) specimens contain fewer A and B1 chains and more B2 and B3 chains than cereal starches. Amylopectin can be decomposed into chains, but this “jigsaw puzzle” is difficult to restore. The amylomaize amylopectin fraction shows intermediate properties between amyllose and amylopectin and will be described later.

9.5.6 MODE OF BRANCHING AND DISTRIBUTION OF BRANCH LINKAGE

For a complete understanding of amylopectin structure, the mode and location of branching should be clarified. The mode of chain linkage in amylopectin can be classified into two types based on the proposal of Saudinger (S-type) and Haworth (H-type), as shown in Figure 9.10. In the former, two chains link to a single chain by their reducing residues, and in the latter a chain links by its reducing residue to the second chain, which links to the third chain by its reducing residue. The structure proposed by Meyer is comprised of both S- and H-types. An enzymatic method of sequential degradation of amylopectin with β-amylase, isoamylase, and α-amylase, called β, i, β-degradation, has been developed to characterize the mode of chain connection and the frequency of A chains on a single B chain [141,214]. For this purpose, B chains are subclassified into Ba and Bb chains. The Ba chain links at least one A chain, and the Bb chain links other B chains but no A chains. First, β-amylase degrades A chains into G2 or G3 residues according to an even or odd number of chain lengths, and B chains are shortened to the outer chains of one or two glucosyl residues. Second, isoamylase hydrolyzes all the branch linkages except maltosyl branch linkages. A small amount of G2 is liberated by isoamylolysis, as mentioned earlier, but less than 10% of the liberation does not cause serious error. Third, β-amylase degrades linear chains originating from B chains. Finally, the maltosyl Ba chains (β, j, β-LD) remain which have half the A chains of the parent amylopectin as maltosyl stubs; from the number of the maltosyl stubs per Ba chain the number of A chains per Ba chain in the amylopectin can be determined. If the Ba chain has one A chain, half of the Ba chain remains as a mono-G2 Ba chain fragment. If the Ba chain has two A chains, half remains as mono-G3-Ba and one fourth remains as di-G2-Ba chain fragments. Similarly, the case of three A chains per Ba chain produces three eighths mono-G2-Ba,
three eighths di-G$_2$-Ba, and one eighth tri-G$_3$-Ba chains. The ratios ($R$) of nonreducing/reducing residue of these β$_j$β-limit dextrins for one, two, and three A chains per Ba chain are 2, 2.33, and 2.71, respectively. The relationship

### TABLE 9.10
Distribution of the Chain Lengths of Some Amylopectins

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Whole</th>
<th>A</th>
<th>B1</th>
<th>B2</th>
<th>B3</th>
<th>B4</th>
<th>Long Chain</th>
<th>A/B1–4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Waxy rice$^a$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CL (max.)</td>
<td>13</td>
<td>19</td>
<td>41</td>
<td>69</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>$\text{Cl}_w$</td>
<td>24</td>
<td>13</td>
<td>22</td>
<td>42</td>
<td>69</td>
<td>101</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Weight (%)</td>
<td>100</td>
<td>50.0</td>
<td>26.2</td>
<td>18.9</td>
<td>4.1</td>
<td>0.8</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Mol%</td>
<td>100</td>
<td>69.2</td>
<td>21.7</td>
<td>8.0</td>
<td>1.0</td>
<td>0.1</td>
<td>—</td>
<td>2.2</td>
</tr>
<tr>
<td>Wheat$^b$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CL (max.)</td>
<td>11</td>
<td>18</td>
<td>40</td>
<td>80</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>$\text{Cl}_w$</td>
<td>25</td>
<td>13</td>
<td>22</td>
<td>43</td>
<td>79</td>
<td>140</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Weight (%)</td>
<td>100</td>
<td>42.7</td>
<td>32.7</td>
<td>16.7</td>
<td>3.2</td>
<td>0.9</td>
<td>4.5</td>
<td>—</td>
</tr>
<tr>
<td>Mol%</td>
<td>100</td>
<td>63.2</td>
<td>28.4</td>
<td>7.5</td>
<td>0.8</td>
<td>0.1</td>
<td>0.0</td>
<td>1.7</td>
</tr>
<tr>
<td>Tapioca$^{ac}$</td>
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<td></td>
</tr>
<tr>
<td>CL (max.)</td>
<td>11</td>
<td>18</td>
<td>38</td>
<td>62</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>$\text{Cl}_w$</td>
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<td>21</td>
<td>42</td>
<td>69</td>
<td>115</td>
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</tr>
<tr>
<td>Weight (%)</td>
<td>100</td>
<td>38.5</td>
<td>32.5</td>
<td>23.0</td>
<td>5.1</td>
<td>0.9</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Mol%</td>
<td>100</td>
<td>59.6</td>
<td>28.7</td>
<td>10.2</td>
<td>1.4</td>
<td>0.1</td>
<td>—</td>
<td>1.5</td>
</tr>
<tr>
<td>Sago $^d$</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CL (max.)</td>
<td>11</td>
<td>14</td>
<td>35</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>$\text{Cl}_w$</td>
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<td>18</td>
<td>43</td>
<td>61</td>
<td>133</td>
<td>—</td>
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</tr>
<tr>
<td>Weight (%)</td>
<td>100</td>
<td>29</td>
<td>46.2</td>
<td>20.3</td>
<td>3.5</td>
<td>0.5</td>
<td>2.6</td>
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</tr>
<tr>
<td>Mol%</td>
<td>100</td>
<td>45.7</td>
<td>44.8</td>
<td>8.4</td>
<td>1.0</td>
<td>0.1</td>
<td>—</td>
<td>0.84</td>
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<td>Kuzu$^e$</td>
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<td></td>
</tr>
<tr>
<td>CL (max.)</td>
<td>13</td>
<td>16</td>
<td>39</td>
<td>72</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>$\text{Cl}_w$</td>
<td>26</td>
<td>13</td>
<td>20</td>
<td>42</td>
<td>70</td>
<td>119</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Weight (%)</td>
<td>100</td>
<td>30.7</td>
<td>42.7</td>
<td>20.2</td>
<td>5.4</td>
<td>1.0</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Mol%</td>
<td>100</td>
<td>47.0</td>
<td>41.9</td>
<td>9.4</td>
<td>1.5</td>
<td>0.2</td>
<td>—</td>
<td>0.89</td>
</tr>
<tr>
<td>Potato$^d$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CL (max.)</td>
<td>16</td>
<td>19</td>
<td>45</td>
<td>74</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>$\text{Cl}_w$</td>
<td>35</td>
<td>16</td>
<td>24</td>
<td>48</td>
<td>75</td>
<td>104</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Weight (%)</td>
<td>100</td>
<td>27.8</td>
<td>34.9</td>
<td>26.0</td>
<td>9.1</td>
<td>2.3</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Mol%</td>
<td>100</td>
<td>44.2</td>
<td>38.1</td>
<td>14.0</td>
<td>3.1</td>
<td>0.6</td>
<td>—</td>
<td>0.79</td>
</tr>
</tbody>
</table>

$^a$ Data from Hizukuri [172].

$^b$ Data from Hizukuri and Maehara [141].

$^c$ Errors in the references are corrected.

$^d$ Data from Takeda et al. [176].
shown in Equation 9.23 exists between the number \(N\) of A chains per Ba chain in amylopectin and the \(R\) value of the \(\beta_i,\beta\)-LD:

\[
R = \frac{\sum_{i=1}^{N} n_i G_2^{(1+i)}}{2^N - 1}
\]  

(9.23)

Accordingly, from analysis of the \(R\) values on the fractionated \(\beta_i,\beta\)-LD by SEC, the distribution of A chain per B chain can be estimated. The structure of \(\beta_i,\beta\)-LD and its relationship to the amylopectin structure are shown in Table 9.11. These results conform only with the Meyer structure, indicating that the chains link in a random fashion within a cluster. The \(\beta_i,\beta\)-LD of wheat amylopectin linked 1.1 to 1.9 \(G_2\) residues per Ba chain fragment and 1.3 on average (Table 9.12), suggesting that the chain carries 1.2 to 3.4 A chains in amylopectin and 2 on average, and that long Ba chains link up to 4 A chains. The smallest \(\beta_i,\beta\)-LD fraction of DP 7.9 carried 1.1 \(G_2\) stubs. The chain length

---

**TABLE 9.11**

<table>
<thead>
<tr>
<th>(\beta_i,\beta)-LD</th>
<th>Haworth</th>
<th>Staudinger</th>
<th>Meyer</th>
<th>Whelan</th>
</tr>
</thead>
<tbody>
<tr>
<td>Structure</td>
<td>(G_2)</td>
<td>(G_2) (n) (G_2)</td>
<td>(G_2) (n) (G_2)</td>
<td>(G_2) (n) (G_2)</td>
</tr>
<tr>
<td>Amount</td>
<td>Trace</td>
<td>Large</td>
<td>Medium</td>
<td>Medium</td>
</tr>
</tbody>
</table>

*See Figure 9.8.*
of the Ba chain was 5.7, and the chain linked 1.2 A chains (N) in amylopectin; therefore, the average span length (SL) was calculated to be 2.7 from Equation 9.22. Span length is the number of (1→4)-α-linkages between the nearest branch linkages:

$$SL = \frac{CL_{of \: Ba \: of \: \beta-LD} \times 1.5}{N}$$  \hspace{1cm} (9.24)

The SL of the amylopectin was found to be in the range of 2.7 to 10.9. Similar results have been reported on waxy rice starch [214]. These results suggest that amylopectin has both heavily and sparsely branched domains. The heavily branched domains are plausibly considered to be noncrystalline and the sparsely branched domains crystalline; therefore, the distribution of the branch linkage seems largely random but not truly random. In addition, from the ratios of A:Ba (2.0:1) and A:B (1.26:1), the ratio of Ba:Bb was found to be 1.7:1, which means that 37% of B chains have no A chains and carry only B chains.

An alternative method, \(\gamma\)-degradation, has also provided evidence for irregular chain connections and multiple branching on a B chain. The preliminary results have been described elsewhere [215]. Potato amylopectin links small amounts of phosphate ester at the C-6 and C-3 positions of the anhydroglucose residues. These phosphate esters block glucoamylase action at or one residue before the phosphorylated residue and produce \(\gamma\)-limit dextrin.

<table>
<thead>
<tr>
<th>Property</th>
<th>Unit</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>Whole</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\beta)-LD</td>
<td>Mo%</td>
<td>16.6</td>
<td>17.4</td>
<td>29.8</td>
<td>36.2</td>
<td>100</td>
</tr>
<tr>
<td>DPn</td>
<td>43.1</td>
<td>24.2</td>
<td>13.5</td>
<td>7.9</td>
<td>18.9</td>
<td></td>
</tr>
<tr>
<td>R</td>
<td>2.9</td>
<td>2.4</td>
<td>2.2</td>
<td>2.1</td>
<td>2.3</td>
<td></td>
</tr>
<tr>
<td>G stub/Ba</td>
<td>1.9</td>
<td>1.4</td>
<td>1.2</td>
<td>1.1</td>
<td>1.3</td>
<td></td>
</tr>
<tr>
<td>Ba CL</td>
<td>39.5</td>
<td>21.2</td>
<td>11.5</td>
<td>5.7</td>
<td>16.3</td>
<td></td>
</tr>
</tbody>
</table>

Amylopectin

<table>
<thead>
<tr>
<th>Property</th>
<th>Unit</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>Whole</th>
</tr>
</thead>
<tbody>
<tr>
<td>A/Ba chain</td>
<td>3.4</td>
<td>2.4</td>
<td>1.6</td>
<td>1.2</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td>Span length (SL)b</td>
<td>10.9</td>
<td>7.8</td>
<td>5.6</td>
<td>2.7</td>
<td>6.9</td>
<td></td>
</tr>
</tbody>
</table>

\(a\) Nonreducing/reducing residue.

\(b\) See Equation 9.24.

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no or several glucosyl stubs linked with (1→6)-α-linkage. These B chains are degraded into phosphorylated or neutral, linear, and glucosylated chains. The linear and glucosylated neutral chains in γ, i-LD provide evidence for the presence of Haworth and Staudinger types of linking, respectively, as shown in Table 9.13. Accordingly, from the structure of γ, i-LD one can deduce the modes of chain connections in amylopectin. The neutral γ, i-LD provides more relevant information than do phosphorylated chains because the phosphates are not evenly distributed in a molecule and the action of glucoamylase stops at phosphorylated sites but not at branch points. The linear chains and glucosylated chains of γ, i-LD provide the evidence for Staudinger and Haworth connections, respectively, and a mixture of both implies a random Meyer structure. The glucosyl stubs on a B chain are the marks of A or B chains linked to the B chain; therefore, from the number of the glucosyl stubs (N) and CL of the B chain one can determine the number of chains linked on the B chain, and the SL can be calculated from the number of glucosyl stubs and the CL of the B chain by the following equation:

\[
SL = \frac{B \cdot CL - 1}{N + 1}
\]  

The results of this experiment were that the span lengths were determined to be up to 12 and that the mode of chain connection was a mixture of Haworth and Staudinger types; this finding supports a Meyer structure, and a minimum unit structure of amylopectin in which a B chain carries 0 to 3 A or B chains (Figure 9.11) has been proposed.

---

**Table 9.13**

<table>
<thead>
<tr>
<th>Modes of Chain Connection</th>
<th>γ, i-LD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Phosphorylated</td>
</tr>
<tr>
<td>Haworth</td>
<td>P</td>
</tr>
<tr>
<td></td>
<td>P</td>
</tr>
<tr>
<td></td>
<td>PP G</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>PP G</td>
</tr>
<tr>
<td></td>
<td>(G)↓</td>
</tr>
<tr>
<td></td>
<td>(G)↓</td>
</tr>
<tr>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: P, phosphate ester at C-3 or C-6; G, glucose; Ø, reducing residue; —, (1→4)-α-linkage.
9.6 AMYLOSE STRUCTURE

9.6.1 HISTORICAL SURVEY

Amylose was initially found to be a linear molecule because it was hydrolyzed completely into maltose and had one reducing and nonreducing terminal residues, as reported by Meyer [216] and supported by other investigators [217]. It is now recognized, however, that some amylose molecules have several branches. The initial recognition of amylose as having a linear nature appears to come from adulterated β-amylase with weak α-amylase, which was difficult to remove completely at the time. In addition, analytical methods with high sensitivity and accuracy for reducing and nonreducing terminal residues and branched linkages were not available. Branching or anomalies in amylose were first suggested by Peat et al. [218,219]. They found that amyloses of potato and other sources were hydrolyzed only partially (about 70%) with sweet potato β-amylase, which was first crystallized by Balls [220], but they observed a greater degree of hydrolysis by using amorphous soybean β-amylase. They thought that the soybean preparation contained an auxiliary enzyme, termed Z-enzyme, which released the anomalous structure. The nature of the Z-enzyme was suggested to be similar to that of β-glucosidase [221], but this idea was not supported [111,222–224], and after long debate it was concluded that the substance was a weak α-amylase [225–227].

One possible reason for incomplete hydrolysis with β-amylase could be the presence of oxidized glucose residues introduced artificially by oxygen at high temperatures during fractionation [228]. Although the chemical nature of this anomaly has not yet been clarified, this artifact does not appear to be responsible under usual fractionation conditions. This effect may cause a less than 10% decrease in β-amylolysis [111]. Moreover, carefully prepared amylose under oxygen-free atmosphere also shows incomplete hydrolysis. Some workers tried to prove that amylose might have a limited number of branches. Potter and Hassid [229,230] measured the molecular weight by osmotic pressure using acetylated amyloses of various plant sources and also terminal
residues by periodate oxidation. Their results indicated that the amyloses had 0 to 2.3 branches and DPn values varying from 620 (Easter lily) to 1300 (tapioca). Neufield and Hassid reported similar results [224].

Kerr and Cleveland [231] estimated branch linkages from the rate of hydrolysis of amylose using glucoamylase and β-amylase that hydrolyzed exowise from nonreducing terminal residues and suggested the presence of one to two nonreducing residues for potato, two to three for tapioca, and none for corn amylose. The methods used by these workers do not seem to be sensitive enough to draw definite conclusions because the “probable concentration of such branch-points is beyond detection by chemical methods” [111]. The evidence for the presence of some (1→6)-α-branch-linkages was provided by increased hydrolysis using β-amylase with the cooperative action of debranching enzymes, yeast isoamylase [232], or bacterial pullulanase [233]. Misaki and Smith [234] suggested the presence of a branch linkage in every 300 and 400 glucose units in corn and potato amyloses, respectively, based on Smith degradation with the methylation technique [235]. This method produces optically active 1-O-methylerythritol from the branched residues.

9.6.2 General Properties

Each amylose from the various plant sources is a heterogeneous mixture of molecules having variable molecular size and branching. Several lines of evidence for the heterogeneity have been provided, such as the fact that aqueous leaching of starch granules at increased temperatures extracts more amylose [236] with increased molecular size and a lower β-amylolysis limit [105,237], and fractional precipitation by increased alcohol concentration results in increased molecular weights [238].

Amylose has a BV from 1.35 to 1.50, IA from 19 to 21, and λmax (iodine coloration) of 640 to 660 nm. These values are often used for the characterization of amylose but are not so useful for determining purity, linearity, or molecular size because of the low sensitivity of the method of determination. Amylose is nearly free from esterified phosphate and binds less than 5 ppm phosphorus. The limit of β-amylolysis of whole amylose is usually in the range of 70 to 85%, as reported by Banks and Greenwood [111], and is related to the proportion of linear and branched molecules, amounts and locations of branch linkages, and chain length of the side chains. These properties of amyloses from various sources are listed in Table 9.14. The properties of β-limit dextrins are similar to those of the parent amylose, except for the increased number of branch linkages and slightly lower IA and BV, because the linear fraction and the outer chain of the branched molecule are hydrolyzed and all the branch linkages remain. The molecule size of the β-limit dextrins is often similar to or even slightly greater than that of the parent amylose, as the linear fraction is smaller than the branched fraction [134].
<table>
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</table>
9.6.3 Molecular Size

The molecular weight and its distribution are characteristic of amylose source. The DPn and DPw of amyloses from various sources have been found to be 700 to 5000 and 2000 to 7000, respectively, with some exceptions as shown in Table 9.15. Cereal amyloses are generally smaller molecules than those of other origin, especially amylomaize amylose. Small molecules have a higher β-amylolysis limit.

The molecular size is usually in the range of 200 to 20,000 DP, although a few exceptions exist. The tuber and root amyloses have fewer small molecules than cereal amyloses. An exception is kuzu amylose, with a size similar to those of cereals. The amylose from sago starch, which has a high viscosity by the Brabender criteria, was found to have a high molecular weight [176]. Generally, the distribution curve of amylose by SEC is monomodal [67,161] and tends to tail toward the lower molecular side, occasionally having a shoulder. Sometimes a minor peak or tailing fraction appears before the main peak in SEC, indicating contaminant amylpectin [67]. The trimodal distribution of water chestnut amylose is a rare case [210]. These fractions of water chestnut suggest that large molecules have more branch linkages, lower β-amylolysis limits, higher blue values, and higher λmax than small molecules. Similar trends have been confirmed with rice and corn amyloses, as shown in Table 9.16 [239]. In general, large amylose molecules contain more branched linkages than do small molecules.

The proportion of linear and branched molecules is calculated by the ratio of the number of branch linkages of amylose to that of β-LD according to Equation 9.26:

\[
Bf = \frac{\text{NC of amylose} - 1}{\text{NC of } \beta\text{-LD} - 1} = \frac{\text{No. of branch linkages of amylose}}{\text{No. of branch linkages of } \beta\text{-LD}} \times 100
\]

Alternatively, the fraction of branched molecules (Bf) is determined by measuring the reduction of branched molecules directly by titration of whole amylose with NaB\(^3\)H\(_4\) followed by hydrolysis with β-amylase. This treatment hydrolyzes linear molecules completely, but branched molecules remain as \(^3\)H-β-LD. Because β-LD and maltose are easily separable, Bf is determined as the ratio of the radioactivities of β-LD and whole amylose:

\[
Bf = \frac{\text{Radioactivity of } \beta\text{-LD}}{\text{Radioactivity of amylose}}
\]
A linear relationship has been found between the limit of $\beta$-amylose and the fraction of branched molecules (Bf) on seven rice amyloses [135]:

$$
\text{Bf} \, (\%) = -1.57 \cdot \beta_{a.1.} + 161 \quad (r = 0.94)
$$

Extrapolation of this line suggests that the $\beta$-amylolysis limit of the branched amylose is 39%, which implies that the $\beta$-amylolysis limit is a function of the number of branched molecules and that the branched structure is similar in all specimens. The relationship is modified as in Equation 9.29:

$$
\text{Bf} \, (\%) = -1.54 \cdot \beta_{a.1.} + 163 \quad (r = 0.71)
$$

for specimens from 26 sources, and the $\beta$-amylolysis limit of the branched amylose is considered to be approximately 41%, which is a little lower than that of amylopectin. The $\beta$-amylolysis limit of branched amylose could be regarded generally as 40%. The decreased branching and lower $\beta$-limit value compared to amylopectin suggest that the branching mode of amylase is different than that of amylopectin. The amount of branched molecules is usually 30 to 50% of whole amylose. Water chestnut amylose containing only 11% branched molecules is characteristic [210]. The number of branch linkages of branched amylose can be determined by measuring the nonreducing residues of the $\beta$-limit dextrans. From the $\beta$-amylolysis limit and the molecular weight (or DP) of the $\beta$-limit dextrin, the molecular weight of the branched component of the amylose can be calculated. From these values and the degree of polymerization of whole amylose, the DP of linear components can be obtained from Equation 9.30:

$$
\overline{\text{DP}}_n \, (\text{linear}) = \left(100 \cdot \overline{\text{DP}}_n \, (\text{whole}) - N_b \cdot \overline{\text{DP}}_n \, (\text{branched})\right)/N_l \quad (9.30)
$$

where $N_b$ and $N_l$ denote the mol% of the branched and linear molecules, respectively. The weight fractions of the branched and linear molecules are calculated by Equations 9.31 and 9.32, where $W_b$ and $W_l$ denote the wt% of the branched and linear molecules:

$$
W_b \, (\%) = \overline{\text{DP}}_n \, (\text{branched}) \times N_b / \overline{\text{DP}}_n \, (\text{whole}) \quad (9.31)
$$

$$
W_l \, (\%) = 100 - W_b \quad (9.32)
$$

The branched fractions of several rice amyloses were found to be 1.8 times larger than the linear fraction, and their amounts were approximately one third (by mole) and one half (by weight) those of whole amylose [135].
<table>
<thead>
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<th>Source</th>
<th>β-Limit (%)</th>
<th>Branch Molecule (mol%)</th>
<th>Number of Branch Linkages</th>
<th>DPn</th>
<th>DPw</th>
<th>Apparent DP Distribution</th>
<th>[η] (ml/g)</th>
<th>Ref.</th>
</tr>
</thead>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
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<td>70</td>
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<td>12.6</td>
<td>4100</td>
<td>5430</td>
<td>840–19,100</td>
<td>—</td>
<td>112</td>
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<tr>
<td>Koganesengan</td>
<td>73</td>
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<td>—</td>
<td>4100</td>
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<tr>
<td>Minamiyutaka</td>
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<td>4400</td>
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<tr>
<td>Norin2</td>
<td>73</td>
<td>—</td>
<td>9</td>
<td>—</td>
<td>3400</td>
<td>—</td>
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<td>334</td>
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<tr>
<td>Yam, Nagaimo</td>
<td>86</td>
<td>29</td>
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<td>—</td>
<td>2000</td>
<td>—</td>
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<td>—</td>
<td>314</td>
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<tr>
<td>Tapioca</td>
<td>75</td>
<td>42</td>
<td>6.8</td>
<td>16.1</td>
<td>2660</td>
<td>6680</td>
<td>580–22,400</td>
<td>384</td>
<td>112, 161, 322</td>
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<tr>
<td>Lotus</td>
<td>90</td>
<td>38</td>
<td>6.7</td>
<td>—</td>
<td>4200</td>
<td>8040</td>
<td>520–42,000</td>
<td>426</td>
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<tr>
<td>Arrowhead</td>
<td>80</td>
<td>—</td>
<td>5.8</td>
<td>—</td>
<td>2840</td>
<td>7080</td>
<td>570–21,300</td>
<td>427</td>
<td>317</td>
<td></td>
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</tr>
<tr>
<td>Bracken (&lt;i&gt;Pteris aquillinum&lt;/i&gt; L.)</td>
<td>77</td>
<td>—</td>
<td>4.2</td>
<td>—</td>
<td>1990</td>
<td>3800</td>
<td>670–8500</td>
<td>315</td>
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<td>Lily</td>
<td>89</td>
<td>39</td>
<td>3.9</td>
<td>10.1</td>
<td>2310</td>
<td>5010</td>
<td>360–18,900</td>
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<td>160, 319</td>
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<td>Edible canna</td>
<td>83</td>
<td>21</td>
<td>2.2</td>
<td>—</td>
<td>1380</td>
<td>5480</td>
<td>550–14,400</td>
<td>361</td>
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<td>Potato</td>
<td>Eniwa</td>
<td>80</td>
<td>—</td>
<td>6.3</td>
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<td>4920</td>
<td>6360</td>
<td>840–21,800</td>
<td>384</td>
<td>112, 161</td>
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<tr>
<td></td>
<td>Jaga Kids Purple 90</td>
<td>85</td>
<td>—</td>
<td>3.9</td>
<td>—</td>
<td>2190</td>
<td>5400</td>
<td>590–12,150</td>
<td>370</td>
<td>179</td>
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<tr>
<td></td>
<td>Jaga Kids Red 90</td>
<td>87</td>
<td>—</td>
<td>3.9</td>
<td>—</td>
<td>2110</td>
<td>5130</td>
<td>560–11,750</td>
<td>368</td>
<td>179</td>
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</table>

<sup>a</sup> 22.5°C, 1-N NaOH.
<sup>b</sup> LV and HV refer to low and high viscosity by Brabender amylograph.
TABLE 9.16
Properties of the Fractions of Maize and Rice Amyloses and Their 
β-Amylese and Isoamylase Limit Dextrins

<table>
<thead>
<tr>
<th>Subtraction</th>
<th>Maize</th>
<th></th>
<th></th>
<th>Rice</th>
<th></th>
<th></th>
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<tr>
<td></td>
<td>F1</td>
<td>F2</td>
<td>F3</td>
<td>F1</td>
<td>F2</td>
<td>F3</td>
</tr>
<tr>
<td>Amylose amount (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Weight</td>
<td>31</td>
<td>38</td>
<td>31</td>
<td>34</td>
<td>36</td>
<td>30</td>
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<tr>
<td>Mole</td>
<td>9</td>
<td>25</td>
<td>66</td>
<td>14</td>
<td>20</td>
<td>66</td>
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<td>Blue value</td>
<td>1.37</td>
<td>1.39</td>
<td>1.14</td>
<td>1.40</td>
<td>1.37</td>
<td>1.34</td>
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<tr>
<td>λmax (nm)</td>
<td>649</td>
<td>642</td>
<td>614</td>
<td>652</td>
<td>645</td>
<td>626</td>
</tr>
<tr>
<td>DPn</td>
<td>2720</td>
<td>1280</td>
<td>390</td>
<td>2230</td>
<td>1670</td>
<td>410</td>
</tr>
<tr>
<td>DPw</td>
<td>6410</td>
<td>1740</td>
<td>570</td>
<td>6280</td>
<td>1930</td>
<td>680</td>
</tr>
<tr>
<td>DPw/DPn</td>
<td>2.36</td>
<td>1.36</td>
<td>1.46</td>
<td>2.82</td>
<td>1.16</td>
<td>1.66</td>
</tr>
<tr>
<td>CL</td>
<td>275</td>
<td>435</td>
<td>250</td>
<td>330</td>
<td>520</td>
<td>295</td>
</tr>
<tr>
<td>Branch linkage/mol</td>
<td>8.9</td>
<td>1.9</td>
<td>0.6</td>
<td>5.8</td>
<td>1.5</td>
<td>0.4</td>
</tr>
<tr>
<td>β-Amylese limit (%)</td>
<td>75</td>
<td>89</td>
<td>90</td>
<td>81</td>
<td>89</td>
<td>92</td>
</tr>
<tr>
<td>Isoamylolysis limit (%)</td>
<td>98</td>
<td>79</td>
<td>50</td>
<td>97</td>
<td>87</td>
<td>75</td>
</tr>
<tr>
<td>Molar fraction</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Linear molecules</td>
<td>0.34</td>
<td>0.66</td>
<td>0.71</td>
<td>0.39</td>
<td>0.64</td>
<td>0.75</td>
</tr>
<tr>
<td>Branched molecules</td>
<td>0.66</td>
<td>0.34</td>
<td>0.29</td>
<td>0.61</td>
<td>0.36</td>
<td>0.25</td>
</tr>
<tr>
<td>β-Limit dextrin</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DPw</td>
<td>5090</td>
<td>1510</td>
<td>500</td>
<td>5120</td>
<td>1820</td>
<td>520</td>
</tr>
<tr>
<td>DPw/DPw (amylose)</td>
<td>0.79</td>
<td>0.87</td>
<td>0.88</td>
<td>0.82</td>
<td>0.94</td>
<td>0.76</td>
</tr>
<tr>
<td>Branch linkage/mol</td>
<td>13.5</td>
<td>5.6</td>
<td>2.1</td>
<td>9.5</td>
<td>4.2</td>
<td>1.6</td>
</tr>
<tr>
<td>Isoamylolysol dextrin</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DPn</td>
<td>335</td>
<td>605</td>
<td>320</td>
<td>395</td>
<td>645</td>
<td>330</td>
</tr>
<tr>
<td>DPn/DPn (amylose)</td>
<td>0.12</td>
<td>0.47</td>
<td>0.82</td>
<td>0.18</td>
<td>0.39</td>
<td>0.80</td>
</tr>
<tr>
<td>DPw</td>
<td>4620</td>
<td>1630</td>
<td>540</td>
<td>4960</td>
<td>1820</td>
<td>600</td>
</tr>
<tr>
<td>DPw/DPw (amylose)</td>
<td>0.72</td>
<td>0.94</td>
<td>0.95</td>
<td>0.79</td>
<td>0.94</td>
<td>0.88</td>
</tr>
<tr>
<td>Branch linkage/mol</td>
<td>0.2</td>
<td>0.4</td>
<td>0.3</td>
<td>0.2</td>
<td>0.2</td>
<td>0.1</td>
</tr>
<tr>
<td>β-Amylese limit (%)</td>
<td>82</td>
<td>91</td>
<td>94</td>
<td>90</td>
<td>93</td>
<td>98</td>
</tr>
<tr>
<td>Short-chain fraction (wt%)</td>
<td>7</td>
<td>2</td>
<td>1</td>
<td>5</td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>


### 9.6.4 Branched Structure

For analysis of the branched structure of amylese, specimens must be confirmed free of amlopectin. The specimens are purified by repeated recrystallization from aqueous 1-butanol, ultracentrifugation, and gel filtration, and purity is confirmed by gel filtration [112]. Ultracentrifugation is necessary to remove large amlopectin, which behaves like a microgel. This large amlopectin can be detected by gel filtration, but small amlopectin is difficult to detect and
should be removed by repeated crystallization. Würsch and Hood [204] detected the liberation of about 1% of the fraction of DP 15 to 20 from mango amylose with isoamylase, and they thought that this fraction originated from low-molecular-weight amyllopectin rather than being chemically bound to the long amylose chain. Also, based on a decrease (15 to 20%) in the limiting viscosity number and a brief change of the Sepharose 4B chromatogram after debranching, they concluded that the side chains have a degree of polymerization of several hundred. Banks and Greenwood [111] concluded that the amylose linked long side chains from the hydrodynamic properties of amylose and its β-LD. The presence of long and short side chains in potato amylose was suggested by the shift of the gel-filtration pattern slightly toward the low-molecular-weight side and the production of oligosaccharides over G₅ by isoamylolysis [112]. Generally, debranching with isoamylase produces small amounts of short-chain fractions as well as high-molecular-weight fractions with a distribution close to that of the original amylose [239]. The liberation of short chains by debranching is only several percent by weight but they are the majority by number. The short side chains of rice [134] and maize [239] amylose fractions liberated with isoamylase, after removal of long chains by precipitation with butanol, are linear molecules with DP of approximately 18.

The side-chain distribution of rice amylose has been revealed by gel-permeation chromatography of ³H-labeled side chains prepared by reduction with NaBH₄ after isoamylolysis of reduced amylose with NaBH₄ [240]. As shown in Figure 9.12, side chains were distributed widely, with degrees of polymerization ranging from 10 to 4000 and a peak at DP 21. Clearly, the

![Figure 9.12 Side-chain distribution of rice amylose. (Adapted from Takeda, Y. et al., Carbohydr. Res., 246, 267, 1993.)](image)
majority of side chains were short, or less than 100 DP; however, long chains were also found. These long side chains, LS1 and LS2, were not entirely linear, and they contained 84% and 38% branched molecules by mole, respectively. This suggests that some long side chains also have side chains, and the reason why these side chains are not liberated by isoamylase requires further investigation. The molar ratio of the very long (DP > 200), long (30 to 200), and short (10 to 30) side chains was about 4:3:20. It is clear that predominant side chains are short, such as in amylopectin.

To explore this heterogeneity and clarify the fine structure, corn amylose was fractionated into two fractions, soluble and insoluble, in the presence of 1-butanol at 40°C [68]. The soluble fraction was minor (2.7% from starch) and gave higher DPw (5910) and lower DPn (580) values than the insoluble fraction (DPw 3880, DPn 800) (16.7%, from starch); also, the soluble fraction demonstrated a bimodal distribution by SEC while the insoluble fraction distribution was monomodal. The soluble fraction liberated 12% short chains (soluble in 10% 1-butanol), but the insoluble fraction liberated only 1%. The short chains had a DP of approximately 18. Isoamylolysis slightly decreased the large molecules, increased the small molecules of the soluble fraction, and produced short chains. These facts indicated that corn amylose is comprised of three branched molecules with high (8930), medium (5700), and low (530) DPw values and has both long and short chains.

Colonna and Mercier [136] suggested the presence of long side chains rather than short chains in wrinkled-pea amylose with two to four chains, similar to smooth-pea amylose, after comparing the chromatograms of native and isoamylase debranched amylose on Sephadex CL-2B. Their chromatogram of debranched amylose on Sephacryl S-200 revealed the presence of a long tailing fraction with a small peak fraction (7.4%). The small peak fraction suggests the presence of a considerable amount (by mole) of short side chains; therefore, their results seem to be consistent with our conclusions [112,151]. However, these authors reported that amylose could be debranched completely by isoamylase alone, which is not consistent with our results. Most amylose specimens are hydrolyzed completely by simultaneous or sequential hydrolysis with β-amylase and pullulanase, but isoamylase alone hydrolyzed only 18 to 95% of branch linkages. The successive actions of isoamylase and pullulanase also could not attain complete hydrolysis [40], and the reason for this may be the presence of a single glucosyl stub.

Abe et al. [38] recovered 63 to 79% of the branch linkages in amylose from branched oligosaccharides, panose, branched tetraose, and branched pentaose by hydrolysis with periplasmic α-amylase from Xanthomonas campetris K 11151, providing evidence for branch linkages in amylose [241]. The same enzyme produced more than 90% of these branched oligosaccharides from amylopectin. The model structures of linear and branched rice amylose are shown in Figure 9.13.
9.7 INTERMEDIATE MOLECULE STRUCTURE

9.7.1 INTRODUCTION

The presence of materials intermediate between amylose and amylopectin has been observed. The criteria for classification are the degree of branching and the molecular weight; however, the limits of these properties of amylose and amylopectin are vague, and no effective method for separation of the intermediate material has been established. The concept of an intermediate molecule, therefore, is hazy, and it is difficult to isolate pure material. The amylopectin fraction of high amylose starches appears to comprise a typical intermediate material in terms of both branching and molecular weight. Some amylopectins link long chains of similar size to amylose and consequently have higher iodine affinities and blue values than normal amylopectin [134]. These molecules also could be defined as hybrid-type intermediates. The branched amyloses with up to 20 or more branch points on average may be intermediate; however, hybrid-type and branched amyloses have not been recognized as being intermediates, because their properties are similar to those of amylopectin and amylose, respectively. An assumed intermediate material that formed a loose layer over a hard amylose–pentanol or –butanol complex in the first fractionation of sweet potato starch was found to be amylopectin [184].

9.7.2 AMYLOPECTIN FRACTION OF AMYLOMAIZE STARCH

The amylopectin fraction of amylo maize starch was first suggested by Wolff et al. [242] to have intermediate properties because it has longer outer and inner chains than those of normal maize. Whistler and Doane [243] reported the presence of a material with intermediate IA and molecular weight properties as determined by precipitation with 2-nitropropane from the amylose fraction of ae and sm2 maize varieties. Mercier found that the amylopectin had longer inner chains (DP ~ 60) than the amylopectin of normal maize (DP ~ 30) based on the chain-length distributions of the amylopectin and its β-LD; also, no short-chain amylose was detected [244]. Greenwood et al.
suggested that this anomalous amylopectin was a mixture of short linear amylose (DP ~ 100) and normal amylopectin (CL of 25). This result was based on the greater yield of glucose than from normal amylopectin by digestion of the amylopectin with excess amounts of β-amylase, as theoretically glucose is produced only from the reducing residues of linear molecules with odd numbers of glucosyl residues.

Baba et al. [247] fractionated amylomaize starch (Amylon 70) into precipitate (P, corresponding to amylose in normal starch) and supernatant (S, amylopectin) by 1-butanol precipitation. After debranching, SEC on Sephacryl G150 revealed that the P and S fractions, as well as the original starch, had three peaks: FI (corresponding to amylose), FII (corresponding to the long chains of amylopectin), and FIII (corresponding to the short chains of amylopectin). From the yields of FI, FII, and FIII; the CL of FII and FIII; the ratio of the amount of FII and FIII; and other data, the authors concluded that the amylomaize starch contained approximately 55% intermediate material, 20% amylomaize-specific amylopectin, and 25% amylose. They could not, however, properly separate and identify the structures of the intermediate material and amylomaize-specific amylopectin.

Wang et al. [248] tried to separate intermediate materials from the amylopectin fraction of several maize mutants by SEC on Sepharose CL-2B. They assumed that the intermediate fraction, which eluted after amylopectin, gave lower ratios of FIII to FII (the referred fraction is denoted as FII/FI) and lower [η] values than those of amylopectin fractions; however, both amylopectin and the intermediate fraction of ae and hybrids with the ae gene showed intermediate properties, while the intermediate fractions of btl and dul had much lower [η] than those of Ap but a high ratio of FIII to FII, similar to amylopectin. Takeda et al. [174] fractionated the amylopectin fraction (supernatant of 1-butanol precipitation) from Hylon 5, Hylon 7, and an unknown variety into four subfractions of similar amounts (FL, FM, FSa, and FSB) by SEC on Toyo-Pearl HW-75 and HW-55SF. The corresponding fractions of these amylomaize starches showed similar properties. As summarized in Table 9.17, the IA, BV, λmax, CL, and ECL of these fractions increased with decreasing molecular weight. The FL fraction with the largest molecular weight seemed to have properties closer to normal amylopectin but gave higher CL and IA values than normal maize. The FM and FSa fractions showed intermediate properties in every respect. The smallest fraction (FSb) contained nearly one branch linkage on average. Some molecules could be linear, as suggested previously [245,246], and others might be considered branched short amylose; therefore, it has been concluded that the amylopectin fraction of amylomaize starches is a heterogeneous mixture of several kinds of molecular species, mainly intermediate materials, with minor amounts of amylopectin and branched and linear amyloses with low degrees of polymerization.
Wang et al. [213] estimated the intermediate materials of 17 maize mutants with the Oh43 inbred line by differences in the fractions of FII (amylose and intermediate) in native starch and FI (amylose) in debranched starches [249] as 15.3% (ae), 15.2% (dul), 9.3% (sul), 22.5% (ae bt1), 18.9% (ae dul), and 11.7% (dul sul), compared with 3.2% in normal maize. Starches with only the ae genotype, however, had longer B chain lengths and considerably lower chain ratios of FIII (A and short B chain) to FII (long B chain) than those of normal and waxy starches. Similar results were reported by Fuwa and his coworkers [133]. A hybrid maize, ae wx, comprised of 95 to 100% amylopectin fraction [250] with an FIII:FII ratio (0.8) less than that of ae (1.0), suggests that the whole granule is intermediate [144].

### 9.7.3 Intermediate Materials from Other Sources

A similar low-molecular-weight intermediate material was separated by differential centrifugation from the amylopectin fraction of wrinkled-pea starch [136]. This material was found to be 29% in wrinkled pea but only 0.45 in smooth pea and had IA values from 8.7 to 9.56, $\lambda_{\text{max}}$ value of 580 nm, $\beta$-amylolysis limit of 70%, and $[\eta]$ of 19 ml/g. These properties are characteristic of intermediate materials. The chain-length distribution showed a small amount

---

**TABLE 9.17**

Properties of the Subfractions from Amylomaize (Hylon 7) Amylopectin Fractions

<table>
<thead>
<tr>
<th>Property</th>
<th>FL</th>
<th>FM</th>
<th>FSa</th>
<th>FSb</th>
<th>Whole*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight proportion (%)</td>
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<td>32</td>
<td>15</td>
<td>27</td>
<td>100</td>
</tr>
<tr>
<td>Iodine affinity, I₂ (g/100 g)</td>
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<td>4.43</td>
<td>7.23</td>
<td>9.57</td>
<td>4.63</td>
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<tr>
<td>Blue value</td>
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<td>0.370</td>
<td>0.445</td>
<td>0.655</td>
<td>0.441</td>
</tr>
<tr>
<td>$\lambda_{\text{max}}$ (nm)</td>
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<td>574</td>
<td>574</td>
<td>580</td>
<td>575</td>
</tr>
<tr>
<td>DPn</td>
<td>—</td>
<td>—</td>
<td>1330</td>
<td>92</td>
<td>—</td>
</tr>
<tr>
<td>DPw</td>
<td>—</td>
<td>19.200</td>
<td>2110</td>
<td>120</td>
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<tr>
<td>DP distribution</td>
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<td>230–13,400</td>
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<tr>
<td>Average chain length (CL)</td>
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<td>By Smith degradation</td>
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<td>34</td>
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<td>49</td>
<td>32</td>
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<tr>
<td>By isoamylosis</td>
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<td>$\beta$-Amylolysis limit (%)</td>
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<td>74</td>
<td>61</td>
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<td>12</td>
<td>15</td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td>Number of chains</td>
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<td>—</td>
<td>32</td>
<td>1.9</td>
<td>—</td>
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</tbody>
</table>

* Yield 58% from starch [174].
(2.1%) of trimodal peaking at the void volume of the Sephacryl S-200 column, and a main-portion DP at 45 (FII) and 15 (FIII). The ratio of FIII to FII was 3.6, while the amylopectin fractions of both wrinkled and smooth peas were approximately 9.6 and 8.1, respectively. The CL of the intermediate material was 28.9, and that of amylopectin was 20.5. This intermediate material may be a heterogeneous mixture of several molecular species, like that of amylo- maize [174]. Bertoft [27] suggested that the intermediate material of the wrinkled pea has an increased proportion of long chains with decreasing molecular weight due to structural changes of the β-limit dextrin during hydrolysis with α-amylase. An appreciable portion of the amylopectin fraction of smooth pea was suggested to be intermediate because it has a low molecular weight and increases the long-chain component [28]. The amylopectin fractions of high amylose rice starches were found to have higher amounts of the long B chain and the intermediate chain than normal rice [132, 251] and could be intermediates, but they have not been well characterized. Oat starches were also reported to have intermediate materials [252]. Intermediate materials seem to cover a broad range with regard to the degree of branching and molecular size, and accordingly it is difficult to separate them.

9.8 STARCH GRANULE STRUCTURE

9.8.1 GENERAL FEATURES

Starch granules have characteristic features dependent on shape, size, and submicroscopic structure. Leaf starches, which are produced directly by photosynthesis, are generally the smallest (less than 1 µm) whereas the root starch of edible canna can be as large as 150 µm. Normal starches contain 16 to 28% amylose (Table 9.5). Pure waxy rice starches are 100% amylopectin, but some specimens contain up to 3 or 5% amylose, perhaps as contaminants from normal varieties. Some barleys are referred to as waxy, but their starch contains up to 10% amylose [253, 254]. High-amylose varieties of maize and rice have 30 to 70% amylose. Starch granules are microcrystalline, comprised of crystalline and noncrystalline domains, and may have some transitional regions. Crystalline domains are believed to be constructed mainly by A chains and outer B chains. Amylose is perhaps in a noncrystalline state.

9.8.2 DOUBLE HELICAL STRUCTURE

Kainuma and French [255] first suggested that the crystalline orientation was built up with double helices, which was possible by both parallel and antiparallel arrangements. From this special model, they suggested that the repeat period of approximately 10.5 Å was comprised of three glucosyl residues that were in good agreement with the observed fiber period. The average diameters of the double helix of crest-to-crest and trough-to-trough arrangement were
suggested to be 1.22 nm and 0.83 nm, respectively, with no room for accommodation of water molecules or other small molecules in the center. The double helical structure has been widely accepted, but, based on the work of Senti and Witnauer [257], who used solid-state $^{13}$C-NMR spectroscopy and x-ray diffraction of the spontaneous transformation of dry V-amylose (left-handed single helix) to the B type in a humid desiccator, Saito et al. [256] proposed that the B type must be the left-handed single helix because the transformation should be caused by an unfolding and refolding process if the B type were the double helix. It was difficult, however, to understand that this process could occur just by humidification.

The crystalline domains of double helices are compact and less susceptible to acid than random-oriented noncrystalline domains; therefore, crystalline domains can be obtained by heterogeneous acid hydrolysis of starch granules. Nägeli amylodextrin is thus prepared by steeping the granule in acids at room temperature for some months. After about 50% is solubilized, the hydrolysis slows. The remainder is enriched by crystalline domains, according to the sharpness of the x-ray diffraction lines, and it retains the original crystal type [258]. Two kinds of molecules were found in Nägeli amylodextrin: (1) DP 12 to 16 linear or glucosyl- or maltosyl-stubbed molecules having a branch linkage near the reducing residue, and (2) DP 28 to 30 molecules with two chains of DP 14 to 16 which form double helices about 5 nm long [259]. The branch linkages in the amylodextrin indicated that some branch linkages were nearby in double helices and might assist the formation of the double helices. The side chain could fold back onto the main chain and produce the double helix, which has been suggested by conformational analysis of the branched tetrasaccharide, $6^2$-α-glucosylmaltotriose, as a model compound of the branching [260]. The amylodextrin was prepared for only 2 or 3 days at 65°C by steeping the starch granules in 70% ethanol containing 1-N HCl, which caused about fivefold more rapid solubilization than H$_2$SO$_4$ [261].

Robin et al. [171] observed the progressive appearance of two major molecules (DP 25 singly branched and DP 15 linear) during linnterization of potato starch, and the former fraction finally changed to the latter. The latter was considered to be generated from crystalline domain of the granule. Based on the structure of amylodextrin, a cluster structure was proposed [171].

### 9.8.3 Crystalline Polymorphs and Their Formation

Starch granules from various botanical sources have one of three x-ray diffraction patterns (A, B, and C) [262]. Most cereal starches, normal maize, rice, wheat, and oats exhibit the A type, referred to as the cereal type. Potato, lily, canna, and tulip starches exhibit the B type, referred to as the tuber type. This classification is inadequate, however, because there are many exceptions. Some root starches, such as taro, some sweet potatoes, tapioca, and iris [184,263,264], exhibit the A type; in addition, some cereals, such as amylo maize, high-amylose barley [265],
and high-amylose rice starches \[132,251\], exhibit the B type. The A and B types are believed to be independent, but it has been suggested that the C type is a mixture of A and B types in various proportions \[264\]. Several rhizome and bean starches belong to the C type. C types have been subclassified as Ca, Cb, and Cc on the basis of their resemblance to A and B types or between the two types, respectively \[266\]. Figure 9.14 shows typical A, B, and C diffraction patterns.

Hizukuri et al. have investigated the causes for the formation of polymorphs by the crystallization of amylodextrin. Amylodextrin is easily dissolved in water and crystallized into the same structures as in native starch granules but gives much sharper x-ray diffraction than starch and its structure is easy to distinguish. Hizukuri et al. found that higher temperatures and concentrations of the crystallization shifted the resulting structure toward the A type \[267\]. Amylodextrin yielded any of three crystalline forms in a narrow temperature range near room temperature, implying that the environmental temperature had a considerable effect on the formation of the polymorphism. This temperature effect was confirmed in growing soybean seedlings and sweet potatoes. Soybean contains no starch, but it produces some starch in the cotyledon during germination, producing B type when germinated at 13.5°C, Cc type at 22°C, and A type at 28°C \[268,288\]. Sweet potato grown at 20°C gave Cc type and at 30°C A type \[270\]; however, this variation did not occur on ripening rice kernels and growing potato tubers at different temperatures \[271\]. Starch produced by \textit{Chlorella vulgaris} was also independent of the temperature \[272\]. These observations imply that the temperature effect is not a primary factor but a modifier. It has been suggested that several anions and cations stimulate the formation of A type by lyotropic order \[266\]. Only \(\text{SO}_4^{2-}\), which induced a B-type formation, was an exception. Alcohols and fatty acids with increased C numbers were stimulated to produce A type \[273\].

\[\text{FIGURE 9.14} \ X\text{-}ray \text{diffraction} \text{patterns} \text{of} \ A, B, \text{and} \ C \text{type.}\]
and phospholipids extracted from wheat [273] and hotwater extracts of various plants also induced A type [274]. These facts suggest that a wide variety of materials have some effect on polymorphism. In particular, lipid seems to affect the formation of A type in cereals; however, amylomaize and wrinkled-pea starches, which contain relatively abundant lipids and possibly are produced in media containing higher concentrations of lipids, exhibit B type.

The CL of amylopectin has been found to be a basic factor for determining the crystalline polymorphs, judging from analysis of amylopectin CL values from wide sources [263]; for example, A-type starches had shorter chain lengths (CL ≤ 19.7) than B-type starches (CL ≥ 21.6), and starches with amylopectin CL values between 20.3 and 21.3 exhibited A, B, or C type. The CL values, in this case, did not include ELCs, and the CL values were slightly lower for specimens containing this fraction. The ELCs seem to have no effect on polymorphism. Furthermore, crystallization of amylodextrin has provided the supporting evidence that amylodextrin with CL values of 13.3, 11.5, and 10.8 crystallized into B, C, and A type, respectively, at 29.5°C [263]. Because the β-amylolysis limits are similar for all amylopectins, the OCL appears to be the key factor for polymorphism. Furthermore, Pfannemüller [275] found that homogeneous short-chain amylose with chain lengths between 10 and 12 crystallized the A type and amylose with chain lengths between 13 and 18 crystallized the B type, and she concluded that B or A polymorphism primarily depended on the CL. Her findings support the concept that the C type is a mixture of A and B types rather than an independent, individual species, because the A or B type was formed from pure amylodextrins differing by one glucosyl length and none of the C type appeared. The A type is suggested to be less hydrated than the B type, as described below, and favorable conditions for the production of A type would include less hydration; thus, the conditions for A and B type formation are consistent with the hydration states of these structures. Still unexplained, however, is why a difference of one or two glucose unit lengths results in a significant difference in hydration, as indicated in A- and B-type structure.

9.8.4 Crystalline Structure

Several unit cell structures of A and B types have been proposed based on powder x-ray diffraction and fiber period. In 1972, French and Kainuma [255] proposed a double helical structure and a monoclinic cell (a₀ = 1.2, b₀ [fiber axis] = 10.48, c₀ = 16.25, β = 96.5°) accommodating two double helices for B-starch. Wu and Sarko presented the models for A-type [276] and B-type [277] structures based on x-ray diffraction data and computerized molecular modeling by using the double helix concept. They concluded that the right-hand, parallel-strand double helices were packed in an antiparallel fashion in both A and B types, but the A and B types differed in their packing modes. This conclusion has been questioned by several investigators, mainly from a biosynthetic standpoint [278,279], who argue that parallel packing would be
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more reasonable. The A-type [280] and B-type [281] structures that would satisfy this condition have been presented based on x-ray and electron diffractions and on computerized molecular modeling by French investigators [282]. Table 9.18 summarizes the unit cell structures for A and B types for which the double helices are essentially identical but the mode of packing the helices and the content of water differ. The double helices are left-hand, parallel-strand structures and are packed in a parallel manner. The O-6 groups are in a position gauche to O-5 and C-4 conformation. Hydrogen bonds are found only in interstrands between O-2 and O-6, which tightly bind the double helices. As shown in Figure 9.15, the double helices of both types are packed in hexagonal

**TABLE 9.18**

Unit Cell Structures of A- and B-Type Starches

<table>
<thead>
<tr>
<th>Unit Cell</th>
<th>A Starch&lt;sup&gt;b&lt;/sup&gt;</th>
<th>B Starch&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dimensions (nm)</td>
<td>( a = 2.124 )</td>
<td>( a = b = 1.85 )</td>
</tr>
<tr>
<td>( b = 1.172 )</td>
<td>( c = 1.04 )</td>
<td></td>
</tr>
<tr>
<td>( c = 1.069 )</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>( \lambda = 123.5^\circ )</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Space group</td>
<td>B2</td>
<td>P6&lt;sub&gt;3&lt;/sub&gt;</td>
</tr>
<tr>
<td>Glucose&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Repeating unit</td>
<td>Maltotriose</td>
<td>Maltose</td>
</tr>
<tr>
<td>Symmetry</td>
<td>Twofold screw axis</td>
<td>Right-handed threefold screw axis</td>
</tr>
<tr>
<td>Water</td>
<td>4 (3.6%)</td>
<td>36 (25%)</td>
</tr>
</tbody>
</table>

<sup>a</sup> Left-handed, parallel-stranded double helices: gauche–gauche conformation.

<sup>b</sup> Data from Imberty et al. [280].

<sup>c</sup> Data from Imberty [281].

**FIGURE 9.15** Packings of double helices (hexagon) in A- and B-type starches. A side of the hexagon is one glucosyl length and the dots indicate water molecules. For a and b, see Table 9.18. (Adapted from Imberty et al. [280,324].)
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or pseudohexagonal arrays. The A type accommodates densely packed double helices, while B-type double helices are loosely packed. The A type contains 4 water molecules per 12 glucose residues, while the B type has 36 water molecules (i.e., 3.6% for the A type and 25% for the B type). These polymorphs have been identified by $^{13}$C cross-polarization and magic-angle spinning (CP/MAS) NMR [283,284]. The A type showed three peaks of C-1 resonance at 99.3, 100.4, and 101.5 ppm, while the B type showed two peaks at 100.0 and 100.9 ppm. These peaks were not easily distinguishable in native starches but were clearly observed on amylopectin with high crystalline quality, as revealed by x-ray diffraction, or on acid-degraded starches. The C-1 spectral difference between the A and B types is consistent with the different helix packing in the A and B types, as there are four different C-1 sites in the A type and two different C-1 sites in the B type with respect to the adjacent double helices [284]. $^{13}$CMNR spectra of A-, B-, and V-type (single helix) starch or amylose together with $\alpha$- and $\beta$-cyclodextrins have been assigned by Gidley and Bociek [283], as shown in Table 9.19.

### 9.8.5 Crystalline Size and Crystallinity

The x-ray diffractograms of starch granules are of poor quality, indicating that the crystalline domains are not well developed. The crystalline size of potato starch was estimated to be 15.0 nm by line broadening of x-ray diffraction [285]. Later this figure was corrected to 13.0 and 12.0 nm on 1.58-nm and

<table>
<thead>
<tr>
<th>Material</th>
<th>C-1</th>
<th>C-4</th>
<th>C-3</th>
<th>C-2,5</th>
<th>C-6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amylose hydrate</td>
<td>103.5</td>
<td>83.2</td>
<td>75.7</td>
<td>73.1</td>
<td>62.2</td>
</tr>
<tr>
<td>Amylose helical complex with Sodium palmitate ($V_6$)</td>
<td>103.6</td>
<td>82.2</td>
<td>75.9</td>
<td>72.7</td>
<td>61.0</td>
</tr>
<tr>
<td>Hexanoic acid ($V_6$)</td>
<td>103.5</td>
<td>82.7</td>
<td>75.9</td>
<td>72.9</td>
<td>61.9</td>
</tr>
<tr>
<td>1-Butanol ($V_6$)</td>
<td>104.1</td>
<td>83.5</td>
<td>75.8</td>
<td>73.5</td>
<td>62.0</td>
</tr>
<tr>
<td>tert-Butyl alcohol ($V_3$)</td>
<td>103.9</td>
<td>83.2</td>
<td>75.7</td>
<td>72.9</td>
<td>61.9</td>
</tr>
<tr>
<td>1-Naphthol ($V_6$)</td>
<td>104.9</td>
<td>84.1</td>
<td>75.4</td>
<td>73.4</td>
<td>61.7</td>
</tr>
<tr>
<td>A-type amylodextrin</td>
<td>101.5, 100.4, 99.3</td>
<td>76.0</td>
<td>70–75</td>
<td>70–75</td>
<td>62.1</td>
</tr>
<tr>
<td>B-type amylodextrin</td>
<td>100.4, 100</td>
<td>76.2</td>
<td>70–75</td>
<td>70–75</td>
<td>62.3</td>
</tr>
<tr>
<td>Amylose (solution)</td>
<td>100.9</td>
<td>78.6</td>
<td>74.6</td>
<td>72–73</td>
<td>61.9</td>
</tr>
<tr>
<td>$\alpha$-CD (solution)</td>
<td>102.6</td>
<td>82.4</td>
<td>74.5</td>
<td>72–73</td>
<td>61.7</td>
</tr>
<tr>
<td>$\beta$-CD (solution)</td>
<td>103.1</td>
<td>82.3</td>
<td>74.3</td>
<td>72–73</td>
<td>61.7</td>
</tr>
</tbody>
</table>


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0.522-nm diffraction lines, respectively [286]. The previous slightly larger value seems to be due to a minute error in the standard quartz use. Broadening of the diffraction lines occurs below 32% water content. At 15.5% water content, the dimensions were found to be 8.7 and 8.0 nm from the 1.58-nm and 0.522-nm diffraction lines, respectively. This is consistent with the structure of the B-type, which contains abundant water [281]. Sterling [287] found, by x-ray small-angle scattering, that the long-range periodicities in various wet starch granules were 9.9 nm for potato, 10.9 nm for normal maize, 10.4 nm for waxy maize, and 10.7 nm for smooth pea, but 0 for wrinkled pea. Hizukuri also observed similar periods: 10.0 nm in wet (42% water content) potato and 11.0 nm in wet normal maize and sweet potato [286]. These scattering patterns were diffused and very sensitive to water content. At a water content of 32%, the long period could no longer be observed, but the wide-angle x-ray diffraction maintained was almost as sharp as that at 40% water content. Sweet potato and maize also had diminished periodicity at 16% water content but still showed sharp wide-angle x-ray diffraction patterns.

These observations indicate that the amorphous domains retain abundant water and preferentially release on drying, so the long period is disturbed. Blandhard et al. [288] also reported the presence of similar periods (10.0 nm) in potato, wheat, millet, barley, waxy maize, mung bean, and smooth pea. Jenkins et al. [289] observed a repeat period of 9 nm in wheat, potato, tapioca, rice, corn, and barley starches by using small-angle x-ray scattering. These periods are thought to be the distance between two crystalline domains and correspond to the average size of an amylopectin cluster, consistent with the cluster size estimated by the CL distribution [172], as 10 nm corresponds to 29 glucosyl residues calculated from the crystalline structures.

Crystallinity has been estimated by some investigators based on the intensity of x-ray diffraction and accessibility of deuterium. Zobel and Senti [290] reported the crystallinities of various starches, determined by x-ray diffraction, as follows: potato, 25%; wheat, 36%; rice, 38%; maize, 39%; and amylomaize, 19 to 24%. The crystallinity of air-dried potato starch (20 to 23%) increased to 28% by moistening and further increased to 34% by lintnerization and moistening [291]. Amylomaize starch was reported to have less crystallinity of 24 and 25% by x-ray diffraction and deuterium exchange method, respectively [292]. Slightly lower values were obtained from x-ray diffraction for wheat (20%), maize (27%), potato (24%), waxy maize (28%), and tapioca (24%) [293]; similar values from deuterium accessibility were reported [294]. The crystallinity of native starch would be within 20 to 40%.

Gelatinized starch gives a diffused halo spectrum by x-ray diffraction, indicating that it is amorphous, although some cereal starches show weak diffraction lines due to the amylose–lipid complex (V pattern). The gelatinized amorphous starch showed a characteristic $^{13}$C-CP/MAS NMR spectrum in which the C-1 peak region (102 to 105 ppm) shifted slightly to low field
compared to that of crystalline material (94 to 105 ppm); the C-4 peak also shifted to 81 to 83 ppm (at least 6 ppm shifted downfield from the crystalline material), and a specific peak for amorphous material shifted to 94 to 98 ppm. Spectra of the native starch show the mixed pattern of crystalline and amorphous materials. From a comparison of standard specimens mixed with various proportions of crystalline and amorphous materials and native starches, the amounts of crystalline domain were estimated to be 42% in maize, 50% in potato, 49% in rice, 53% in waxy maize, and 38% in amylomaize [294]. These data are considerable higher values than those estimated by x-ray diffraction. The higher values can be explained by the two states of double helices (non-crystalline and crystalline) in starch granules [293]. The $^{13}$C-CP/MAS NMR findings correspond to both double helices but the x-ray diffraction corresponds only to the crystalline organization of the double helix. $^{13}$C-CP/MAS NMR is a molecular sensor, and x-ray diffraction is a crystalline sensor. The different results obtained by the two methods indicate that some double helices are not oriented in a crystalline order.

### 9.8.6 Amorphous Domain Structure

The major part of starch granules is believed to be an amorphous gel phase, in which several materials, such as free amylose (F-amylose), lipid-complexed amylose (L-amylose), and mainly a frequently branched portion of amylpectin, are mixed together. The conformation of the chains appears to be a single helix or random coil. In addition, some double helices are also thought to be present as described above.

Normal cereal starches contain approximately 1% lipids. Lysophospholipids are the main components, particularly in wheat, barley, rye, and triticale. Free fatty acids make up one third or one half of the lipids in normal maize and rice [120]. Waxy maize and rice starch have only about one tenth the lipids of normal species. Amylomaize starch contains about 50% more lipids than maize, and the major components are free fatty acids [295]. These lipids have been thought to be helical complexes with amylose in the granules, because these lipids are barely extractable with common fat solvents such as diethyl ether and chloroform [124,125]. It has been suggested that the difficulty in extracting lipids from starch granules is due to the resistant nature of the starch granule, because lipids have been rapidly extractable from amylose–fatty acid complexes at high temperatures (98 to 100°C) and efficiently even at ambient temperature by various polar solvents [296]. The difficult extraction, therefore, may not be evidence for the presence of amylose–lipid complexes in the granule. Some amylases are easily extractable from granules with water at slightly above gelatinization temperature to approximately 80°C, and this water-extractable amylose has been increased by preliminary defatting [297]. Because the amylose–lipid complexes are
insoluble in water and their dissociation temperatures are above 93°C [298],
the water-extractable amylose appears to be noncomplexing free amylose (F-
amylose) and the remainder lipid-complexing amylose (L-amylose).

No evidence for the presence of amylose–lipid complexes in native starch
granules has been obtained by x-ray diffraction, indicating that these com-
plexes are not organized into the crystalline state but are buried separately in
the amorphous domains or exist in too small amounts. The presence of an
amylose–lipid complex of approximately 16.0 nm in wheat starch has been
suggested by small-angle neutron scattering [288]. Evidence for the presence
of an amylose–lipid complex has been provided by 13C-CP/MAS NMR [253].
A barley starch, containing higher amylose (32.7%) and lipids (969 mg as
lisophospholipid per 100 g), showed a weak resonance with a chemical shift
of 31.2 ± 4 ppm that is characteristic of midchain methylene carbons of solid-
state fatty acids of the V-amylose complex. The concentration of the midchain
carbon deduced from the intensity of the peak was 0.3 ± 0.05 mol%, which
was comparable to that (0.4 mol%) calculated from the lipid content of the
starch. The amylose–lipid complex was acid resistant, and the resonance peak
was intensified by the acid hydrolysis of granular starch [156,253]. In waxy
and normal barley starches, 96.3% of lipid-complexed amylose or 72.1% of
free amylose was suggested to remain during lintnerization together with
double helical regions of outer chains of amylopectin [156]. This is explained
by the specific hydrogen bonding between OH-3’ and OH-2 of consecutive
glucosyl residues of V-type structure [299,300].

Supporting evidence for the presence of amylose–lipid complexes in native
starch granules was also provided by differential scanning calorimetry. Mor-
ris and coworkers [253] measured the gelatination enthalpy of 12 varieties
of waxy barley (amylose content, 1.7 to 7.4%) and six varieties of normal
barley (amylose content, 29.2 to 32.7%). The values were corrected for the
amount of amylopectin, because the endothermic gelatinization was caused
by disorganization of the double-helical crystalline domains of the molecules.
The corrected values of enthalpy for amylopectin were constant for all the
specimens regardless of L-amylose content, suggesting no exothermic forma-
tion of an amylose–lipid complex during gelatinization. The amount of L-
amylose is calculated based on the phospholipid content of the starch by
multiplying the amount (%) of lisophospholipid by 7. Approximately 21%
and 54% of the amylose of normal and waxy barley starches were found to
be L-amylose, respectively; thus, two forms of amylose (L-amylose and F-
amylose) appear to be present in barley starches and are probably present in
other starches. These two forms of amylose could be a factor in controlling
the gelatinizing properties of starch granules [253].

Potato starches with a higher degree of phosphorylation were found to
have fewer crystalline domains, suggesting that P6 interferes with crystalliza-
tion because C-3 carbons face the interior cavity of the double helix [301].
Amylose seems to be richer in the periphery than in the center of the granules because BV, λmax, IA, and the FI fraction (amylose) have been reported to increase during the development of starch-storage organs in several plants, such as endosperms of some kinds of maize (except wx) [302,303], wheat [304,305], barley [306], rice [307], pea seeds [308], and potato tubers [309]. On the other hand, the presence of blue-staining cores with iodine in waxy varieties of maize and barley [310] and a decrease in the amylose content of a maize hybrid (ae wx) [303,144] during development suggest a reverse distribution of amylose.

It has been suggested that amylose is randomly interspersed in a single molecule among amylopectin molecules rather than being in bundles, because SEC gave no indication of cross-linkages among amylose molecules but did reveal an increase in the BV of the amylopectin fraction due to crosslinking with epichlorohydrin [311]. This was further confirmed on crosslinked normal cornstarch phosphorus oxychloride by gel-exclusion chromatography and 31P-NMR analysis [187]. Jane and Shen [192] suggested that amylose is relatively abundant at the periphery of potato starch granules due to stepwise chemical gelatinization from the periphery.

9.9 CONCLUSION

This chapter outlined the structures of starch molecules and granules. An excellent article on a similar subject [312] has been published and is helpful for developing a better understanding of the subject. Starch remains a complex material that exhibits characteristic structures and widely varying functions depending on the individual species. Determining the structures of amylose, amylopectin, and the starch granule has been challenging. The relationship between structure and function is of prime importance in both academic and industrial fields. With the recent progress in biochemical and instrumental techniques utilizing enzymes, SEC, NMR, and differential scanning calorimetry, substantial progress has been made, but many topics remain uninvestigated.

ABBREVIATIONS AND SYMBOLS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>BV</td>
<td>Blue value</td>
</tr>
<tr>
<td>CD</td>
<td>Cyclodextrin</td>
</tr>
<tr>
<td>CL and CL</td>
<td>Chain length and average chain length</td>
</tr>
<tr>
<td>CP/MAS</td>
<td>Cross-polarization and magic-angle spinning</td>
</tr>
<tr>
<td>DP, DPN, and DPw</td>
<td>Degree of polymerization, number-average degree of polymerization, and weight-average degree of polymerization</td>
</tr>
<tr>
<td>ELC</td>
<td>Extra (or extended) long chain</td>
</tr>
</tbody>
</table>
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$G_1$, $G_2$, $G_3$, … $G_n$ Glucose, maltose, maltotriose, …, a series of maltooligosaccharides and polysaccharides (starch and glycogen)

HPAEC High-performance anion exchange chromatography
HPLC High-performance liquid chromatography
IA Iodine affinity (g/100 g)
LD Limit dextrin
$M_n$ and $M_w$ Number-average molecular weight and weight-average molecular weight
NC and NC Number of chains per molecule and average NC
NMR Nuclear magnetic resonance
OCL and ICL Average outer and inner chain length
P6 and P3 Esterified phosphates at C-6 and C-3
SEC Size-exclusion chromatography
SL and SL Span length and average span length (span length is the number of $\alpha$-(1→4)-linkages between the nearest branch linkages)
$\alpha$-, $\beta$-, $\gamma$-, and $\phi$-LD $\alpha$-Amylase, $\beta$-amylase, glucoamylase, and phosphorylase limit dextrin
$[\eta]$ Limiting viscosity number (ml/g)
$\lambda_{\text{max}}$ Maximum wavelength of the iodine coloration spectrum

REFERENCES


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24. Yamamoto, T., Bacterial α-amylases (liquefying- and saccharifying-type) of 
\textit{Bacillus subtilis} and related bacteria, in \textit{Handbook of Amylases}, Amylase 
26. Bertolf, E., Studies on the structure of pea starches. 3. Amylpectin of smooth 
27. Bertolf, E., Studies on the structure of pea starches. 4. Intermediate material 
29. Taniguchi, H., Odashima, F., Igarashi, M., Maruyama, Y., and Nakamura, M., 
Characterization of a potato starch-digesting bacterium and its production of 
30. Hayashida, S., Teramoto, Y., and Inoue, T., Production and characteristics of 
raw-potato-starch-digesting α-amylase from \textit{Bacillus subtilis} 65, \textit{Appl. Envi-
31. Umeki, K. and Yamamoto, T., Structures of multi-branched dextrins produced 
by saccharifying α-amylase from starch, \textit{J. Biochem.}, 78, 897, 1975.
32. Mizowaki, K., \textit{Streptococcus bovis} α-amylase, in \textit{Handbook of Amylase and 
Related Enzymes: Their Sources Isolation Methods, Properties, and Applica-
p. 49.
33. Hizukuri, S., Fine structure of starch molecules and the actions of amylases, 
34. Sakano, Y., Higuchi, M., and Kobayashi, T., Pullulan 4-glucanohydrolase from 
35. Shimizu, M., Kano, K., Tamura, M., and Suekane, M., Purification and some 
properties of a novel α-amylase produced by a strain of \textit{Thermoactinomyces 
36. Kuriki, T., Okada, S., and Imanaka, T., New type of pullulanase from \textit{Bacillus 
steathermophilus} and molecular cloning and expression of the gene in \textit{Bacillus 
37. Kim, C.I., Cha, J.H., Kim, J.R., Jang, S.Y., Seo, B.C. et al., Catalytic properties 
of the cloned amylase from \textit{Bacillus licheniformis}, \textit{J. Biol. Chem.}, 267, 22108, 
38. Abe, J., Shibata, Y., Fujisue, M., and Hizukuri, S., Expression of periplasmic 
α-amylase of \textit{Xanthomonas campestris} K-1151 in \textit{Escherichia coli} and its 
action on maltose, \textit{Microbiology}, 142, 1505, 1996.
38a. Abe, J., Onitsuka, N., Nakano, T., Shibata, Y., Hizukuri, S., and Entani, E., 
Purification and characterization of periplasmic α-amylase from \textit{Xanthomonas 
39. Nanmori, T., Bacterial β-amylase, in \textit{Handbook of Amylase and Related 
Enzymes: Their Sources, Isolation Methods, Properties, and Applications}, Amylase 
40. Takeda, Y., and Hizukuri, S., Improved method for crystallization of sweet 
376  Carbohydrates in Food


169. Nikuni, Z., Denpun and chori [starch and cookery], *Chorikagaku*, 2, 6, 1969.


© 2006 by Taylor & Francis Group, LLC
255. Kainuma, K. and French, D., Naegeli amylodextrin and its relationships to starch granule structure. II. Role of water in crystallization of B-starch, Biopolymers, 11, 2241, 1972.


10 Starch: Physicochemical and Functional Aspects

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and Magnus Gudmundsson

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10.1 INTRODUCTION

In this chapter, some physicochemical and functional properties of starch are described. Starch is used in a wide range of products, either as a raw material or as a food additive; therefore, starch plays many roles in food. It works as a thickener, as a gelling agent, as an absorber of water, as a source of energy in fermentation, as a bulking agent, or as an antisticky/sticky agent. Moreover, starch is used in foods with very different water contents, from products such as dressings or still drinks with very high water contents to products such as licorice, which has a very low water content. It is thus evident that the functional properties of starch have many aspects. In this chapter, the most basic physicochemical properties are described to serve as a foundation for an understanding of the various functions of starch.

When starch is heated in water, it absorbs water and swells. This is the process of gelatinization, a process that causes a tremendous change in the rheological properties of the starch suspension. The properties of starch gels are very sensitive to factors such as shearing, temperature, heating or cooling rate, and, of course, the source of the starch and the presence of other components. The gelatinization process depends on the presence of both crystalline and amorphous domains in the starch granule.

The starch component in a food product may cause several problems. A starch gel is not a system in equilibrium; it changes with time. The crystalline structure has been destroyed during gelatinization, but crystallinity can emerge again during storage. The ability of starch molecules to crystallize after gelatinization is referred to as retrogradation. Although retrogradation of amylose seems to be a prerequisite for the formation of a normal bread crumb, retrogradation usually causes a deterioration in quality. This can express itself as syneresis (i.e., loss of water) or as increased hardness. The leaking of amylose from the starch granule during gelatinization causes stickiness, and one way of reducing this stickiness is to add polar lipids (e.g., monoglycerides). The interaction of starch with other components, such as polar lipids and proteins, influences the functional properties greatly.

The modern food industry puts several demands on starch. It should be able to stand high shear rates and shear forces in processing equipment, and it should be able to stand low pH values. It should also tolerate the high temperatures that occur in sterilizing processes, and it should tolerate low temperatures (e.g., during storage in the refrigerator or freezer). In addition, it should also tolerate being heated in a microwave oven when the consumer is preparing the product for consumption at home. Native starches usually do not meet all these requirements; therefore, starch has been modified in different ways. Chemical modifications change the starch molecules through their covalent linkages. Physical modifications involve changes in starch structure or phase behavior without the involvement of covalent linkages. Interactions
between starch and other components (e.g., lipids, proteins) might also be described as physical modifications. Through genetic modification, the composition, in terms of amylose and amylopectin, might also be changed. The aim of all these modifications is to improve the functional properties of starch.

Starch is basically composed of a mixture of amylose and amylopectin. The composition of these starch molecules is fairly simple; they are polymers built up from only one type of monomer — glucose. It is still possible to have great variation in the chemical structures (see Chapter 9) and, as will be clear from this chapter, in the physicochemical and functional properties.

10.2 THE STARCH GRANULE

Various starches differ considerably from each other in properties. It has been suggested that no two granules, even from the same botanical origin, are similar; “in fact, it is probably no exaggeration to say that each granule in a population is unique, differing from its neighbors in terms of both fine structure and properties” [1]. All granules are thus individuals. Still, it is possible to identify properties of a typical starch that put starch in a category of its own among polysaccharides. Starch is found in nature as particles. These particles cause starch systems to behave like suspensions or dispersions. After heating and mechanical treatment in food processing, remnants of the starch particle are left.

The particle, of course, has a surface, and the importance of this surface with regard to interactions between the starch and other components should be given some attention. One example of how the starch surface comes into play is enzymatic digestion. A prerequisite for hydrolysis is that the enzymes are adsorbed onto the starch granule [2]. The hydrolysis may then proceed either through surface erosion or through penetration through pinholes and subsequent hydrolysis from the inside out [3].

The interior of the starch granule is composed of alternating crystalline and amorphous regions. Starch is frequently described as a semicrystalline or partly crystalline polymer [4–7]. The melting of crystallites and disruption of the organized structure are the basis for gelatinization.

10.2.1 THE STARCH PARTICLE

10.2.1.1 Morphological Aspects

The shape of the starch granule depends on the botanical source, and many different forms are found in nature. Also, the size varies, from the tiny granules in rice and oat to the large ones in potato and banana starch. Some typical dimensions for a number of starches are given in Table 10.1. The cereal starches (wheat, rye, and barley) show a bimodal size distribution. The small granules
Starch: Physicochemical and Functional Aspects

(B-granules) are spherical with a diameter below (roughly) 10 µm, and the large granules (A-granules) are lenticular with a diameter around 20 µm.

The particle size distribution of a commercial starch might not reflect the true size distribution in the botanical tissue; for example, depending on the isolation procedure, a smaller or larger proportion of the B-granules may be lost in wheat starch [12,15]. Moreover, separating the various size classes by gravity seldom gives 100% A-granules and 100% B-granules [15a].

TABLE 10.1

<table>
<thead>
<tr>
<th>Starch</th>
<th>Diameter (µm)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dent corn</td>
<td>10.3–11.5</td>
<td>8</td>
</tr>
<tr>
<td>Potato</td>
<td>37.9</td>
<td>9</td>
</tr>
<tr>
<td>Potato</td>
<td>50</td>
<td>10</td>
</tr>
<tr>
<td>Rice</td>
<td>5–6</td>
<td>10</td>
</tr>
<tr>
<td>Taro</td>
<td>2–5</td>
<td>10</td>
</tr>
<tr>
<td>Rice</td>
<td>6.8</td>
<td>11</td>
</tr>
<tr>
<td>Cassava</td>
<td>16.8</td>
<td>11</td>
</tr>
<tr>
<td>English wheat</td>
<td>4.5, 15.3</td>
<td>12</td>
</tr>
<tr>
<td>Canadian wheat</td>
<td>4.0, 14.5</td>
<td>12</td>
</tr>
<tr>
<td>Wheat</td>
<td>6.1–6.3, 18.2–19.3</td>
<td>9</td>
</tr>
<tr>
<td>Durum wheat</td>
<td>5.7–6.2, 18.3–25.0</td>
<td>9</td>
</tr>
<tr>
<td>Barley</td>
<td>3.1–3.7</td>
<td>13</td>
</tr>
<tr>
<td>Normal barley</td>
<td>5, 10–25</td>
<td>14</td>
</tr>
<tr>
<td>Waxy barley</td>
<td>5, 25</td>
<td>14</td>
</tr>
<tr>
<td>Tapioca</td>
<td>17.7</td>
<td>9</td>
</tr>
</tbody>
</table>

For most starches, the granule is formed inside the amyloplast, and one starch granule is inside each amyloplast [16]. In some starches (e.g., oat), many tiny granules (4 to 10 µm in diameter) form aggregates of a much larger size (diameters of 20 to 150 µm) [17]. Also, the small granules in wheat, the B-granules, are reported to have a tendency to agglomerate when separated from the wheat kernel [17a].

Because of the characteristic morphological properties of the granules it is possible to identify most starches from their appearance under a light microscope [18]. The light microscope reveals other features of the starch particle in addition to shape and size. The cereal starches wheat, rye, and barley show an equatorial groove along the large granules [19]. Surface indentations are found in some starches, assumed to be the result of close packing in the cell [20]. Enzyme attacks might be observed as pits in the surface [18,21]. Surface pores or fissures have also been observed without excess

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enzyme activity in rye starch [22], wheat, barley, maize, and sorghum starches, but not in rice, oats, potato, tapioca, arrowroot, or canna [23,24]. It has been suggested that these pores, with diameters of 0.1 to 0.3 µm, are openings to channels that penetrate the interior of the granule, perhaps even into the hilum [24]. Depressions on the surface of cereal starches have also been revealed by noncontact atomic force microscopy (AFM) [24a]. The size of the depressions depends on the botanical source of the starch. Differences in starch granule surface features between potato and wheat starches were observed by AFM [24b], and the wheat starch granule surface was smoother with fewer protrusions than the potato starch surface.

10.2.1.2 Composition

Typical values of the composition of starch granules are shown in Table 10.2. The polysaccharides amylose and amylopectin are the most abundant components and will constitute almost 100% of a typical starch. The ratios between amylose and amylopectin differ between starches, but a typical value for a “normal” starch is 25% amylose and 75% amylopectin (see Table 10.2). In some starches (e.g., maize, barley, rice), genotypes exist with either an increased amylopectin content (waxy varieties) or an increased amylose content (high amylose or amylostarches). Waxy varieties of wheat starch have also been produced [29a]. With regard to potato starch, high-amylopectin starch has been produced through genetic modification [29b,30,31].

The components present in addition to the starch molecules are usually described as “minor components” because they are present in low amounts. Although they are present at very low levels, they have a dramatic effect on physicochemical properties. A protein content below 0.5% is typical, and the lipid content, typical of cereal starches, is usually around 1% (Table 10.2). Phosphate groups are typical of potato starch, and the phosphate content is of the magnitude of 1 phosphate ester per 200 glucose units [32]. The values shown in Table 10.2 are obtained from chemical analysis of extracted starch, and some of the values (especially lipid and protein content) depend on how efficient the washing procedure has been.

As discussed later in this chapter, amylose forms a helical inclusion complex with polar lipids. It has been discussed whether such a complex exists in the native starch granule or whether it is formed during gelatinization. X-ray diffraction analysis has not been able to give an unambiguous answer because if the crystalline domains are too small they will not show up in analysis. Evidence has been obtained that the complexes exist before gelatinization, at least in some starches. The V-pattern can be found in high-amylose starches, in starches containing genes such as the amylose extender gene, and in dull or sugary starches [33]. When 13C-cross-polarization/magic-angle spinning nuclear magnetic resonance (13C-CP/MAS NMR) was used to study the
complexes, it was shown that wheat and barley starches gave the same type of signal as the amylose–lipid complex [34]. When amylose and lipids were present without forming an inclusion complex, no signal was obtained. Although the hydrocarbon chain of the lipid in the complex is in the solid state, the amylose–lipid complex is thought to be present as an amorphous structure [34–36]. These complexes might thus serve as nuclei for crystallization during gelatinization and other heat treatments [33]. Cocryotallization between the amylose–lipid complex and the amylopectin double helix cannot occur; there must be different crystalline domains in a starch gel [37].

The protein present might very well be starch-degrading enzymes, and if the conditions are such that the enzymes become active this will certainly influence the functionality of the starch. Excess enzyme activity is usually not preferred, and, particularly for baking, enzyme activity that is too high can be disastrous. Other proteins have been identified as integral components of the starch granule structure, located either in the interior [38] or at the surface [39]. Examples of the latter type of protein are storage proteins, starch bio- synthetic enzymes (e.g. starch-granule-bound starch synthase), and friabilin/puroindolines [39a].

<table>
<thead>
<tr>
<th>Starch</th>
<th>Amylose (%)</th>
<th>Protein (g/100 g)</th>
<th>Lipids (g/100 g)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cassava</td>
<td>17</td>
<td>0.1</td>
<td>0.1</td>
<td>25</td>
</tr>
<tr>
<td>Potato</td>
<td>21</td>
<td>0.06</td>
<td>0.05</td>
<td>25</td>
</tr>
<tr>
<td>Rice</td>
<td>12.2–28.6</td>
<td>—</td>
<td>0.63–1.11</td>
<td>26</td>
</tr>
<tr>
<td>Waxy rice</td>
<td>0–2.32</td>
<td>—</td>
<td>—</td>
<td>27</td>
</tr>
<tr>
<td>Wheat</td>
<td>28</td>
<td>0.3</td>
<td>0.8</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>29.2</td>
<td>—</td>
<td>0.85</td>
<td>29</td>
</tr>
<tr>
<td>A-granules</td>
<td>28.4–27.8</td>
<td>—</td>
<td>0.67–0.73</td>
<td>29</td>
</tr>
<tr>
<td>B-granules</td>
<td>27.5–24.5</td>
<td>—</td>
<td>0.73–0.91</td>
<td>29</td>
</tr>
<tr>
<td>Barley, waxy</td>
<td>2.1–8.3</td>
<td>—</td>
<td>0.30–0.49</td>
<td>26</td>
</tr>
<tr>
<td>Barley, normal</td>
<td>25.3–30.1</td>
<td>—</td>
<td>0.68–1.28</td>
<td>26</td>
</tr>
<tr>
<td>Barley, high amylose</td>
<td>38.4–44.1</td>
<td>—</td>
<td>1.05–1.69</td>
<td>26</td>
</tr>
<tr>
<td>Oat</td>
<td>25.2–29.4</td>
<td>—</td>
<td>1.35–1.52</td>
<td>26</td>
</tr>
<tr>
<td>Maize, normal</td>
<td>28.7</td>
<td>0.3</td>
<td>0.8</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>25.8–32.5</td>
<td>—</td>
<td>0.61–0.82</td>
<td>26</td>
</tr>
<tr>
<td>Maize, waxy</td>
<td>1.4–2.7</td>
<td>—</td>
<td>0.02–0.14</td>
<td>26</td>
</tr>
<tr>
<td>Maize, high amylose</td>
<td>42.6–67.8</td>
<td>—</td>
<td>1.01–1.09</td>
<td>26</td>
</tr>
<tr>
<td>Fava bean</td>
<td>33</td>
<td>0.9</td>
<td>0.1</td>
<td>28</td>
</tr>
<tr>
<td>Pea</td>
<td>33</td>
<td>0.7</td>
<td>0.1</td>
<td>28</td>
</tr>
</tbody>
</table>
10.2.2 Starch Crystallinity

Starch granule crystallinity can be studied with the x-ray diffraction technique, and four different x-ray diffraction patterns have been described: the A-pattern, obtained for cereal starches (except high-amylose varieties); the B-pattern obtained for root and tubular starches (and for high-amylose varieties) and retrograded starch; the C-pattern, obtained for beans and peas; and the V-pattern, obtained for gelatinized lipid-containing starches. The C-pattern is described as a mixture of the A- and B-patterns but has also been regarded as a structure of its own [33]. In normal starches (i.e., starches with both amylase and amylopectin) and of course in waxy starches it is the branched (amylopectin) molecules that constitute the crystallites. The branches of the amylopectin molecules form double helices that are arranged in crystalline domains [40]. The A-, B-, and C-patterns are thus different polymorphic forms of starch that differ in the packing of the amylopectin double helices. Wheat and rye starches have been reported to contain low levels of B-crystallites (up to 10%), so the starch granules may be even more heterogeneous than has previously been assumed [41]. Because of line broadening in the x-ray diffraction pattern, it can be concluded that the starch crystals (crystallites) are very small and imperfect [42]. The crystallites have been reported to be spherical in wheat starch as well as in potato starch, with diameters of about 10 nm in wheat and somewhat smaller in potato [43]. The level of crystallinity is also rather low; values in the range of 15 to 45% have been reported for several starches [33], and the crystallinity increases with the ratio of amylopectin in the starch [41]. It has been observed that a greater number of double helices is present than the number arranged in crystalline domains [44]. For wheat, the percentage of double helices was 39% and the degree of crystallinity was 20%; for maize, the corresponding values are 43 and 27%, respectively; and, for potato, the corresponding values are 40 and 24% [45]. The degree of crystallinity is very dependent on the water content, and the highest amount of crystallinity is observed at some intermediate water content [7,46].

The formation of A-crystals is favored by a short average chain length in amylopectin, a high temperature of formation, a high concentration, the presence of a salt with a high number in the lyothropic series, or the presence of water-soluble alcohols and organic acids [47]. Transitions from an A-structure to a B-structure through a C-structure have been observed [48]. The polymorphs are thus related to each other in such a way that the A-structure is most stable:

\[
\text{melt} \rightarrow \text{B-structure} \rightarrow \text{C-structure} \rightarrow \text{A-structure}
\]

For isolated crystallites that melt unrestricted, a lower melting temperature has been observed for B-crystallites compared with A-crystallites [49]. Crystalline A-spherulites were found to melt at 90°C and B-spherulites at 77°C.
The dissolution temperature of the B-polymorph of amylose in water is related to the chain length [50a].

In the crystalline and amorphous regions in starch granules, three types of structures can be identified (Figure 10.1): (1) crystalline domains, (2) amorphous regions containing branching points alternating with crystalline domains, and (3) a second amorphous phase surrounding the alternating crystalline and amorphous regions.

Microscopy studies have further elaborated the details regarding the organization of starch granules [50b], and the organization of amyllopectin lamellae in blocklets (20 to 500 nm) has been visualized. The organization of amyllopectin in the crystalline domains has been described using the side-chain liquid-crystalline model for synthetic polymers [50c]. In this model, three components are used to describe the polymer: rigid units, flexible spacers, and a flexible backbone. The model must be linked to the chemical structure of the amyllopectin molecule which is becoming increasingly better understood with time.

Amylopectin constitutes the crystalline domains, with the double helices arranged in the A-, B-, or C-pattern. The branching regions in the amyllopectin molecules constitute the amorphous layer that separates the crystallites from each other. Because of the size of the amyllopectin molecule, the same molecule might take part in several crystalline domains. Surrounding these alternating crystalline and amorphous domains is another amorphous structure,
carbohydrates in food

causing what has been described as “growth rings.” It has been suggested that a Bragg peak at 10 nm, which disappears on gelatinization, is due to the alternating amorphous and crystalline layers [51]. The Bragg peak has been detected around 10 nm for several starches by both x-ray diffraction techniques and optical diffraction [52], although d-spacings at 26 to 30 nm have also been reported [53]. It has also been suggested that the size of the combined crystalline and amorphous parts (9 nm) is a fundamental feature of the packing of starch molecules and is approximately constant for all starches [54]. To account for the spherical shape of crystallites and for the existence of a layered structure, a curved crystal structure has been proposed [55]. The curvature is the result of a translatory displacement of the helices in the parallel packing.

The structure of the amorphous regions is not known. An interesting aspect that has implications for the organization of the starch granule is that incompatibility has been observed for amylose and amylopectin. In aqueous solutions of these components, phase separation into an upper, clear, amylose-rich phase and a lower, opalescent, amylopectin-rich phase was observed after 24 hours at 80°C [56]. For binary amylose–water systems, the introduction of a third component (amylopectin) has always been accomplished by the aggregation of amylose [57].

Chemical modification (crosslinking) has shown that the amylose molecules are not located together in bundles [58]. This conclusion was reached because no crosslinking was observed between amylose molecules, only between amylopectin molecules and between amylopectin and amylose. The latter observation also indicates that amylose molecules might be rather evenly distributed in the starch granule.

Due to the leaking of amylose and amylopectin during gelatinization, it has been suggested that the location of amylose differs among starches. For oat starch, amylose and amylopectin were found to coelute from the granule during heating, whereas in barley starch the amylose was preferentially leached [59]. For wheat starch heated to 95°C, most of the amylose leached out of the granules [60], whereas in potato starch the amylose diffused toward the aqueous center of the granule [61]. For maize starch, it has been observed that annealing causes an increase in the differential scanning calorimetry (DSC) endotherm attributed to the melting of crystalline amylose, indicating that in this starch the location of amylose molecules is such that it is possible to obtain an ordered, crystalline structure [62].

Although the structures of the amorphous phases are largely unknown, they certainly influence the properties of the starch. A higher water content in the B-polymorph, not only in the unit cell but also in the amorphous regions, could contribute to the lower stability of this polymorph [49]. As will be discussed later, a glass transition of the amorphous regions will precede the crystallite melting during gelatinization.
10.2.3 Starch Granule Surface

In light microscopy or in scanning electron microscopy, the starch granule surface looks very smooth and featureless [18,19,63], except for the grooves and fissures described previously in this chapter. The starch granule surface contains the nonreducing end-groups from amylose and amylopectin. The arrangement of starch molecules in the surface is unknown, but it has been described as a “hairy billiard ball” [64]. Analysis of the surface composition of wheat starch granules shows that several elements are present in addition to the carbon, oxygen, and hydrogen that are expected in amylose and amylopectin; for example, nitrogen, phosphorous, sulfur, and chlorine are found in the surface, indicating the presence of protein as well as lipids [65]. Moreover, a quantitative analysis showed that the surface is enriched in nitrogen and phosphorous compared to the interior of the starch granule.

Measurements of the zeta-potential for starch before and after extraction with different solvents showed that the zeta-potential changed in a way that could be explained by the removal of proteins as well as of lipids [66]. Dye-binding experiments also have shown that proteins are present on the starch granule surface [67]. It is possible to extract proteins from the wheat starch granule surface with molecular weights ranging from 5 to 97 kDa [39,68]. It has been suggested that one of these proteins, friabilin (molecular weight of 15 kDa), is related to the endosperm hardness encountered during the milling of wheat [69]. More recently, the presence of proteins and lipids on the starch granule has been verified using confocal laser scanning microscopy [69a], and it has even been possible to identify the fatty-acid-chain composition of the lipids [69b].

Removal of proteins from the starch granule surface seems to change the degradation pattern of amylglucosidase [70]. The protein also influences swelling and the degree of gelatinization at a certain temperature [67,71]. It has been suggested that certain proteins (e.g., granule-bound starch synthase) are important for maintaining the integrity of starch ghosts (i.e., the starch granule envelopes remaining after gelatinization) [69a]. Complete extraction of lipids is difficult to achieve without disruption or gelatinization of the starch granule [72]; nevertheless, partial extraction seems to indicate an increase in swelling and viscosity [73].

The starch granules usually are not completely dissolved in food processing, and the food can be regarded as a suspension or a dispersion in which starch granules or granular remnants constitute a dispersed phase. The presence of the granules thus introduces an interface. The surface area of A-granules is around 0.25 m²/g in wheat starch and 0.7 m²/g in B-granules [12]. This interface governs interactions between starch and other components through its hydrophobic–hydrophilic character and through the components present at the interface. The components might cause changes in the hydrophobic character as
well as allowing specific interactions to occur. The wheat starch granule surface has been described as hydrophilic, whereas chlorination or gelatinization renders it hydrophobic [74]. A nonchlorinated wheat starch has no oil-binding capacity at all in the presence of water. Starch granules are otherwise completely wetted by oil [75]. The influence of wheat starch granules on the rheological properties of dough depends on the surface properties of the granules [75a]. A surface coating with proteins increases the storage modulus ($G'$) compared to an uncoated starch.

The presence of an interface means that surface-active components might adsorb to this interface. For the degradation of starch by enzymes, adsorption of the enzymes to the starch granule surface is a requisite [2]. The adsorption might be prevented by the presence of a protein layer or a protein gel network. In vitro methods for predicting starch availability are improved if a protein-degrading enzyme is included [76]. The adsorption is further prevented in the presence of cyclodextrins such as cycloheptaamylose [77]. The relationship between endosperm hardness and the presence of certain proteins on the wheat starch granule can certainly be a result of protein adsorption onto the starch granule. Wheat storage proteins adsorb to wheat and maize granules and especially to potato starch, whereas bovine serum albumin is adsorbed to a very low extent [78]. When wheat starch is added to a mixture of wheat proteins, the proteins composed of high-molecular-weight subunits will be preferentially adsorbed [79].

### 10.3 Gelatinization

This section deals with the phenomenon of gelatinization. This is a collective term used to describe a range of irreversible events occurring when starch is heated in water; however, as illustrated in Figure 10.2, not all combinations of starch, water, and temperature result in gelatinization. A certain minimum level of water content is necessary, and a certain temperature has to be reached. This temperature depends on the starch. The word “gelatinization” seems to imply that gel formation is involved; however, not all combinations of water content and temperature that are found in the gelatinization area in Figure 10.2 will result in the formation of a starch gel. The rheological changes during gelatinization are described in a following section. Gelatinization is often described with, or related to, a gelatinization temperature. Different methods are used to determine gelatinization temperatures; therefore, values found in the literature differ considerably, even for the same starch. It is then natural to begin this section with a description of what the gelatinization temperature is.

#### 10.3.1 Gelatinization Temperature

The gelatinization temperature is always a temperature range. For a single starch granule in excess water, this temperature range might be 1 to 2°C, whereas for the entire population the range might be 10 to 15°C [80,81]. $T_g$
is the temperature at the onset of gelatinization, $T_m$ is some kind of midpoint temperature (related to the method), and $T_c$ is the temperature when the gelatinization process is completed. Listed in Table 10.3 are examples of events that can occur during gelatinization, together with the temperature interval at different water contents.

Many parameters affect the gelatinization temperature and temperature range, something that will be frequently noted throughout this chapter. Perhaps the most fundamental influence is that of water, because water serves as a plasticizer for the starch crystallites [88]. The presence of water will decrease the temperature of the glass transition ($T_g$) and, as a consequence, the temperature of the melting of the crystallites. Because this event might be regarded as the primary process in gelatinization, all other events will be affected when these temperatures are manipulated (see Table 10.3). Also, the secondary events depend on the water content; an obvious example is the strong dependence of the rheological properties of the starch gel on water content. Our discussion of gelatinization begins with the primary events and what happens when starch is first brought into contact with water.

### 10.3.2 Starch in Water

Although the starch granules are built up from polymers that are hydrophilic, the starch granule itself is not soluble in water due to the semicrystalline structure of the starch granule and the hydrogen bonds formed between hydroxyl groups in the starch polymers. This is essential for the biological
function of the starch granule in a plant; if the energy is dissolved too early, it is not much of an energy store.

Starch granules are not homogeneous with regard to the distribution of water, and the water content at certain locations (e.g., at the site of the crystallites during melting) is not known. Some of the water is located in the

<p>| TABLE 10.3 |</p>
<table>
<thead>
<tr>
<th>Irreversible Events Occurring During Gelatinization and the Onset ($T_o$) and Conclusion ($T_c$) Temperature for Various Water Contents</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Starch</strong></td>
</tr>
<tr>
<td><strong>Appearance of DSC endotherm</strong></td>
</tr>
<tr>
<td>Rice, waxy</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Rice, normal</td>
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<tr>
<td></td>
</tr>
<tr>
<td>Maize, waxy</td>
</tr>
<tr>
<td>Maize, normal</td>
</tr>
<tr>
<td>Amylo maize V</td>
</tr>
<tr>
<td><strong>Disappearance of birefringence</strong></td>
</tr>
<tr>
<td>Maize</td>
</tr>
<tr>
<td>Wheat</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Barley</td>
</tr>
<tr>
<td>Barley, high-amylose</td>
</tr>
<tr>
<td>Barley, waxy</td>
</tr>
<tr>
<td><strong>Disappearance of crystallinity</strong></td>
</tr>
<tr>
<td>Wheat</td>
</tr>
<tr>
<td>Potato</td>
</tr>
<tr>
<td>Maize</td>
</tr>
<tr>
<td>Maize, waxy</td>
</tr>
<tr>
<td><strong>Swelling</strong></td>
</tr>
<tr>
<td>Wheat</td>
</tr>
<tr>
<td>Barley, waxy</td>
</tr>
<tr>
<td>Barley, normal</td>
</tr>
<tr>
<td><strong>Solubility</strong></td>
</tr>
<tr>
<td>Wheat</td>
</tr>
<tr>
<td>Barley, waxy</td>
</tr>
<tr>
<td>Barley, normal</td>
</tr>
</tbody>
</table>

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amorphous parts, but some water is also present in the unit cells of the crystallites [37]. If the starch is dried to complete dryness, the x-ray diffraction pattern disappears [42]. With NMR or DSC it can be observed that a proportion of the water will not freeze during the experimental conditions (i.e., at temperatures at or below –50°C). These values are in the range of 0.25 to 0.40 g water per g starch [89–91].

When dry starch granules are placed in water, a small amount of water is absorbed. This is an exothermic process, and the heat of immersion decreases to zero at a water content of 0.18 g water per g dry starch for wheat and 0.20 g water per g dry starch for potato [42]. These values correspond to the water content of starch in equilibrium with ambient air, which is around 13% (w/w) for wheat and 18 to 22% (w/w) for potato [92]. Expressed in another way, this is 1.5 water molecules per anhydrous glucose unit. Potato starch granules in equilibrium with excess water have a water content of 0.54 g water per g dry starch (i.e., 5 water molecules per anhydrous glucose unit) [80].

If the temperature is increased, the amount of absorbed water increases. Up until a certain temperature (the onset of gelatinization), the water uptake is reversible, but after that the changes are irreversible. For potato starch, the volume of swollen granules can be almost 200 ml/g dry starch [93]. It should be pointed out, however, that it is not the amount of water that is important but the amount of solvent [93a].

10.3.2.1 Loss of Birefringence

In polarized light, ungelatinized starch granules show birefringence, and a typical so-called Maltese cross is more or less evident [18]. When $T_o$ is reached, the birefringence begins to disappear (see Table 10.3). This loss indicates that order is lost in the starch granules, although not necessarily order at the crystalline level. One of the most common methods for determining the gelatinization temperature range is to follow the loss of birefringence in excess water [94]. Viewing a starch-containing food sample in polarized light in a microscope might thus give some indication of the heat treatment that the sample has undergone [95]. The loss of birefringence occurs over a broader temperature interval when the water content is decreased. For wheat starch with 29% (w/w) water heated to 132°C, birefringence has still been detected [96], and with a water content below 8% not even heating to 232°C destroyed the birefringence [97].

10.3.2.2 Loss of Crystallinity

The loss of crystalline order during heating is observed in x-ray diffraction. The diffraction pattern disappears, and eventually a pattern indicative of a completely amorphous material is obtained [98]. In cereal starches, the V-pattern (i.e., the x-ray diffraction pattern related to the amyllose–lipid complex)
is observed after gelatinization [33]. The temperature range during which the crystallinity is lost and the rate at which it is lost depend on the water content and on the type of starch [7,99]. The temperature range increases with decreasing water content, and at a water content below 50% the temperature for complete loss of crystallinity approaches 100°C. The loss of crystallinity seems to occur in two steps. At first, the loss occurs at a very low rate, but then at a temperature typical of the starch the rate increases dramatically [7]. In small-angle x-ray scattering (SAXS) a d-spacing between 260 and 296 Å is observed [53]. This spacing seems to be related to the birefringence and disappears on heating. The Bragg peak that occurs at about 10 nm, thought to be due to the alternating crystalline and amorphous layers, disappears during gelatinization [51,52,54].

10.3.2.3 Endothermic Transitions

Starch gelatinization is an endothermic process, with enthalpy values in the range of 10 to 20 J/g. Waxy wheat starch has higher transition temperatures and enthalpies than nonwaxy starch [99a], but when the enthalpy values were compared on an amylopectin basis they were identical. In a study of ten different starches (including A-, B-, and C-starches), a relation between transition enthalpy and the amylopectin unit-chain distribution was found; that is, the enthalpy increased when the amylopectin unit-chain length increased [99b]. Another trend was a negative correlation between transition temperatures and amylose content. DSC has become perhaps the most important tool for studying starch gelatinization [88,100,101]. A typical DSC thermogram is given in Figure 10.3. Loss of birefringence occurs in about the same temperature interval as the DSC endotherm (see Table 10.3), or at slightly lower temperatures, in excess water [81]. In more concentrated systems, the loss of birefringence has been shown to coincide with the high-temperature part of the double endotherm [97,102].

The relation between the endothermic processes and the loss of x-ray diffraction intensity is also illustrated in Figure 10.3. It is evident that the DSC thermogram shows other thermal events besides the melting of crystallites. The origin of double endotherms at certain water contents has been much discussed since they were first reported [88,100]. A first approach was to treat the melting according to the Flory–Huggins approach — that is, as the equilibrium melting of the polymer crystals (starch crystallites) in the presence of a plasticizer (water). Although this approach gave much insight into the process it is not strictly correct because equilibrium melting is not obtained during the DSC scan. The occurrence of double endotherms has also been explained as being due to a transition between different polymorphic forms of starch [5]; however, x-ray diffraction studies do not support such transitions [7,98].

The suggestion that the melting of crystallites is preceded by a glass transition seems to be a more fruitful approach [49]. The location of $T_g$ is very
dependent on the water content [103]; therefore, the hydration of starch, or perhaps rather the distribution of water inside the starch granule, is of great importance. When starch is placed in excess water at room temperature, the water content inside the granule at equilibrium will be 0.54 g water per g starch [80], which is much lower than the final value of the water-holding capacity, or swelling volume, of the gelatinized starch. It has therefore been suggested that, during the initial part of the gelatinization, endotherm hydration of amorphous parts occurs, causing the $T_g$ to be passed. In excess water, this process, as well as the melting of crystallites, occurs very quickly, whereas at lower water contents both processes occur much more slowly; therefore, they separate into two distinct peaks in the DSC thermogram. At even lower water contents, a situation will finally be reached when no more water is distributed to the amorphous starch and the melting has to occur at the initial water content. The plasticizing effect of water is then low, meaning that the melting temperature of the crystallites is very high. This is exactly what is observed: At water contents below 30% (w/w) only one or no endotherm at all is detected at temperatures below 100°C [88,97,100]. According to the study by Perry and Donald [93a], it is more correct to describe gelatinization as depending on the availability of a solvent. They suggested that gelatinization begins when the molecular mobility in the amorphous regions of the starch granule reaches a critical level. This mobility depends on the total plasticization, which in turn will depend on both the presence of plasticizing solvent (e.g., water) and thermal energy.

The occurrence of glass transition in starch systems has been deduced from a shift in the baseline during heating of an aqueous starch suspension [103–105]. Such a shift in the baseline can be due to a glass transition [5,49], but it may also be due to differences in heat capacity between the solid state (starch granule) and the liquid state (gelatinized starch granule) [104].
The occurrence of a glass transition during the early stages of gelatinization was suggested by results of DSC experiments carried out by heating starch systems to different temperatures during the DSC scans and then cooling and reheating [49], as well as by the finding that the crystallinity disappears stepwise [7]. In some starches, it has been possible to identify the $T_g$ just preceding the gelatinization endotherm [5,106].

### 10.3.2.4 Morphological Changes

The morphological changes occurring when starch is heated in excess water have been studied using the scanning electron microscope (SEM) [19,63]. In some starches (e.g., potato and maize), the granules seem to swell to a similar degree in all directions, resulting in swollen granules that are approximately similar in shape to the original ones, just larger. The size of starch granules during heating has been determined for some maize starches [107]. For waxy maize, the diameter increased from 15.6 to 39.6 $\mu$m at maximum swelling; for normal maize, the corresponding values were 14.9 to 33.3 $\mu$m; and for cross-linked waxy maize, the corresponding values were 14.5 to 31.5 $\mu$m. In another investigation, the diameter of gelatinized starch granules was found to be 46 ± 15 $\mu$m for maize, 34 ± 24 $\mu$m for wheat, and 85 ± 25 $\mu$m for pea [108]. The large standard deviation indicates a broad size distribution of gelatinized granules. For wheat, barley, and rye starches, the swelling is restricted in one dimension, resulting in complicated folding of the granules [63]. The same morphological changes occur, albeit shifted to higher temperatures, when the water content is decreased [109]. For these cereal starches, it should then be possible to determine the degree of heat treatment in a process by studying the shape of the granules.

### 10.3.2.5 Swelling

Disordering of the crystalline domains in the starch granules is thus the first step in gelatinization. Methods such as measuring the loss of birefringence, DSC, or x-ray diffraction are able to measure this process in one way or another. The values for gelatinization temperature ranges measured with these methods usually coincide (see Table 10.3). For the application of starch (e.g., in the food industry), this first step is necessary but is not enough. For the development of useful functional properties, such as water-holding capacity or rheological properties, the events coming after the melting of the crystalline structure are the important ones. It has already been noted that the starch granule absorbs water. This absorption leads to the morphological changes described and to a considerable swelling of starch granules. The swelling is often measured as an increase in gel volume, and some typical results are given in Figure 10.4.

The swelling begins at a temperature corresponding to $T_g$ in DSC measurements, but it continues to much higher temperatures than the $T_g$ [29].
swelling is rapid during the first 5 to 10 minutes at a certain temperature but continues upon further heating [29]; however, the prolonged holding of temperatures at about 95 to 96°C does not seem to increase the swelling to a great extent [28,110,114]. The swelling is not affected by presoaking before heating but does increase with increasing water-to-starch ratios up to 25 ml water per g starch. The swelling was reported to increase as a result of defatting the starch [29], although for oat and pigeon pea starches defatting seems to decrease swelling [111–113].

The heating procedure influences the swelling (e.g., increased shearing has been found to increase the swelling) [28]; however, severe shearing will cause fragmentation of the granules [61]. Another parameter of importance is the heating rate. For maize and wheat starches at 90°C, the swelling ratio was higher for the higher heating rate, the effect being greater for wheat compared with maize [114].

It is evident from Figure 10.4 that different starches show different swelling patterns, with regard to both the final gel volumes obtained and the temperature response. As can be seen from Figure 10.4, potato starch gives a very large gel volume. The waxy varieties show the highest gel volumes when starches differing in amylose content are compared [29,84].

10.3.2.6 Leaking

During heating and at the same time as the absorption of water, material is leached out from the starch granules. The material is largely amylose, although amyllopectin might be leached, the amount depending on the starch and the
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Conditions [29, 61, 108, 110]. Lipids have not been found in the leached material [29]. Some examples of the leaking as a function of temperature are given in Figure 10.5. The material solubilized during gelatinization can be characterized by staining with iodine, either in solution or in preparation for microscopy. The material leached from undamaged potato and cereal starches in the temperature interval 50 to 70°C is mainly composed of amylose. The solubilized material increases in molecular weight and becomes more branched with increasing temperature [28, 115, 116]. In an oat starch suspension heated to 90°C, amylose was found outside the granules, forming a network structure around them [59]. In barley starch, on the other hand, granules were found to still be stained heavily blue at 90°C, whereas at 95°C not much blue staining was found inside the granules. Demixing of amylose and amylopectin was observed in the microscope for potato starch [61]. If the starch contains a proportion of enzymatically or mechanically damaged starch, this will influence the nature of the solubilized material. Extraction of damaged granules with cold water preferentially leaches out amylopectin of low molecular weight [117].

It has been suggested that in maize and wheat starches most of the amylose will be solubilized before leaking of amylopectin begins [110]. In oat starch, on the other hand, a concurrent leaking of amylose and amylopectin seems to occur [111]. In case of oat starch, it was suggested that instead an intermediate material (molecules less branched than amylopectin) is coleached with amylose [112].

**FIGURE 10.5** Leaking of amylose during heating of potato (○), wheat (△), maize (△), and oat (△) starches. (Adapted from Eliasson, A.-C., J. Text Stud., 17, 253, 1986; Eliasson, A.-C., Starch/Stärke, 37, 411, 1985; Doublier, J.-L. et al., Cereal Chem., 64, 21, 1987.)

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The solubility has been found to depend on the heating rate for wheat starch, but not for maize starch [114]. A prolonged holding time at 96°C also increases the solubility [111]. If the mechanical treatment is severe, this will also change the nature of the solubilized material, and the solubility will increase [111,118]. In normal starches (e.g., normal maize) an increased proportion of amylopectin is found in the solubilized material when the stirring increases [84]. Increased stirring increases the leaking also from high-amylose varieties [84].

Not all amylose leaches out during heating. After heating at 90°C, pea starch was found to contain 16% amylose; wheat, 8.3%; and maize, 8.0% [108]. The solubilized material corresponds to only 6 to 9% of the total starch in high-amylose starches but to 60 to 76% in normal starch [84]. The parts of the granules remaining after gelatinization (ghosts) contain mainly amylopectin without any crystalline order [116]. A decrease in amylose content from 27.4% in native barley starch to 6.2% in a ghost preparation was reported.

As will be described later, the leaking of amylose is necessary for gel formation, but in many cases the leaking of amylose causes problems, as is the case for pasta or potato flakes. To avoid free amylose that causes stickiness, monoglycerides or other emulsifiers might be added to form a helical inclusion complex with amylose.

10.3.3 MEANS OF INFLUENCING STARCH GELatinIZATION

There are many means of affecting the gelatinization temperature, both on purpose and unintentionally. This can be achieved through the addition of other food components to the starch, by using genotypes differing in amylose/amylopectin ratios, by chemical modification, and by annealing or other types of heat treatment. In this section, treatments corresponding to Figure 10.2 are described, and other approaches are described in subsequent sections.

Gelatinization is brought about as a consequence of certain combinations of heat and water content; however, when inspecting Figure 10.2 it is evident that water and heat might influence the behavior of starch without causing gelatinization. Here, two different possibilities are investigated: heat–moisture treatment and annealing. In fact, this is a rather artificial classification because the underlying mechanism is more or less the same; however, it has become customary in the literature to refer to treatments at low water contents and temperatures below the gelatinization temperature range as heat–moisture treatments and to treatments involving high water contents at temperatures above the gelatinization temperature range as annealing. A third possibility is the extrusion process, where high temperatures and low water contents are combined with high shear forces due to mechanical treatment. The following discussion is limited to systems without the application of shear forces.
10.3.3.1 Heat–Moisture Treatment

If the water content is lower than that required for gelatinization but the temperature is within what is necessary for gelatinization, or higher, the starch is exposed to what has been described as a heat–moisture treatment [119–122]. If these samples are then gelatinized (i.e., moved to the right in Figure 10.2), their properties change. Some examples of the influence of heat–moisture treatment on physicochemical properties are given in Table 10.4 [87,199–122].

For the starches in Table 10.4, both $T_o$ and $T_c$ have moved to higher temperatures after the treatment. For potato and wheat starches exposed to heat–moisture treatment (16 hr at 100°C, 27% water), a DSC study showed that not only was the gelatinization temperature range broadened and moved to higher temperatures but the biphasic endotherm, normally observed at intermediate water contents, was also observed for the treated samples in excess water [123]. The biphasic endotherm was more evident when the moisture content was high during the treatment (i.e., 27%). The structural changes seemed to be greater in potato starch than wheat starch. This is also evident from the fact that the x-ray pattern is changed (from B-pattern to A-pattern) [123]. For cassava/tapioca, a change from the C-pattern to the A-pattern has been observed due to heat–moisture treatment [120]. The heat–moisture treatment thus causes a change in the type of crystallinity, from the less stable polymorphs (B and C) to the most stable one (A). It has been observed that a transition from the B-polymorph to the A-polymorph might also occur in response to microwave radiation [123a].

Other properties, such as swelling power and solubility, also change. A decrease in these properties has been observed for several starches, including wheat and potato starches [122,124]. The effect was greater for potato starch, meaning that this starch became more similar to wheat starch. The baking performance of the heat-treated potato starch improved somewhat but was still inferior compared with wheat starch [119]. For the latter starch, the baking performance deteriorated with heat–moisture treatment.

In the examples given above, heat–moisture treatments have been applied to starches on purpose; however, such treatment might be expected to occur during processing when conditions as given in Figure 10.2 are met, and these changes may be neither known nor desired. One such process that might influence starch properties is the drying of wheat kernels, which might result in increased $T_m$ values measured by DSC [125] and increased relative crystallinity of the starch [126]. The x-ray pattern, in accordance with the discussion above, is the A-pattern, independent of the drying temperature; however, a new d-spacing appears at 4.4 Å that has been attributed to the V-pattern. It has been suggested that the drying procedure causes the formation of an increased number of amylose–lipid complexes or more crystalline complexes. Also, the growing conditions in the field might influence starch gelatinization temperatures [125a].
10.3.3.2 Annealing

If the water content is high enough for gelatinization but the temperature is too low, the conditions might be suitable for annealing (i.e., a process that improves the crystallinity; see Figure 10.2). The temperature for achieving annealing ($T_a$) must be below $T_m$ or the crystallites will just melt and above $T_g$ or the system will be too rigid for anything to happen. In this temperature range, the least perfect crystallites melt and the molecules crystallize on the other, more nearly perfect crystals. For starch, this means that the same type of crystallites will be obtained, but of better quality and perhaps with a higher degree of crystallinity. If gelatinization then occurs (i.e., see upward movement in Figure 10.2), it will be apparent that the gelatinization temperature range has moved to higher temperatures and has become more narrow. [83,106,127–129].

For large barley starch granules, the modal gelatinization temperature was found to increase from 61 to 74°C after annealing at 50°C for 6 weeks [116]. Such long annealing times are not necessary for an effect. For steeping (i.e., when cereal grains are soaked in water to facilitate the extraction of starch), changes in properties have been observed after 24 to 72 hours [86,87]. When starch is treated, annealing times much less than 24 hours have been reported [83,129]. The important parameter is not time but the difference between $T_m$ and $T_a$. If this difference is 20 to 25°C or more, no influence at all is evident, whereas a difference around 5°C has been observed to cause an increase in $T_o$ of around 13°C for wheat and potato starches [129]. No subsequent leaking of amylose occurs during the annealing [83].

Just as for heat treatment, annealing causes an increase in the onset of gelatinization, but then some interesting differences occur. With annealing, the gelatinization temperature range narrows [83,127,129]. If the annealing is carried out at water contents for which a biphasic DSC endotherm is obtained for the untreated starch, annealing will transform the biphasic endotherm into a single one [129]. Changes in enthalpy ($\Delta H$) after annealing have been reported, but these changes are difficult to interpret; for example, a decrease in $\Delta H$ might indicate that $T_g$ is too close to $T_m$ (i.e., gelatinization has occurred).

A change in the polymorphic form as a result of annealing has not been reported, but a slight improvement of the x-ray diffraction lines and a decrease in the background have been [87,130]. For normal and high-amylose starches, an increase in the V-pattern due to annealing has been reported [87]. Swelling and solubility are both affected by annealing, and usually they are found to decrease [86,87]. An exception has been found in barley starch, for which the swelling was found to increase at 90°C.

Commercial starches might undergo an annealing treatment during the production process. When laboratory-prepared samples of corn starch were compared with commercially produced ones [131], the laboratory-prepared samples showed a broad gelatinization temperature range, whereas the commercial samples showed a rather narrow range. Annealing, however, did not
### TABLE 10.4
Changes in Functional Properties Due to Heat–Moisture/Annealing Treatments

<table>
<thead>
<tr>
<th></th>
<th>Barley (18% Water)</th>
<th>Barley (27% Water)</th>
<th>Potato (18% Water)</th>
<th>Potato (27% Water)</th>
<th>Waxy Maize (72 hr at 40°C)</th>
<th>Normal Maize (72 hr at 40°C)</th>
<th>High-Amylose Maize (72 hr at 40°C)</th>
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<tr>
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<td>62</td>
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<td>65</td>
<td>60</td>
<td>58</td>
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<tr>
<td>60°C</td>
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<td>2.81</td>
<td>2.87</td>
<td>3.00</td>
<td>3.70</td>
<td>2.55</td>
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<tr>
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<td>6.47</td>
<td>5.36</td>
<td>62.3</td>
<td>24.7</td>
<td>19.1</td>
<td>9.23</td>
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<tr>
<td>90°C</td>
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<td>7.71</td>
<td>6.46</td>
<td>90.6</td>
<td>36.6</td>
<td>24.4</td>
<td>9.83</td>
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<td><strong>Solubility (%)</strong></td>
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<tr>
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<td>0.57</td>
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<td>1.95</td>
<td>1.55</td>
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</tr>
<tr>
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<td>1.24</td>
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<tr>
<td>Baking results</td>
<td>Bread volume (ml/g)</td>
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<td>3.95</td>
<td>3.29</td>
<td>3.17</td>
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<td>Score</td>
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<td>Score</td>
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<td>64</td>
<td>36</td>
<td>68</td>
<td>73</td>
<td>85</td>
</tr>
</tbody>
</table>

<sup>a</sup> Heat–moisture treatment: 18 or 27% water, 16 hr at 100°C.
<sup>b</sup> Annealing: 72 hr at 40°C.
<sup>c</sup> WBC, water-binding capacity.
<sup>d</sup> Loaf volume (ml).
<sup>e</sup> Value for untreated wheat starch.

Source: Data from References 87, 119–122.
very much affect the commercial samples, whereas the laboratory-prepared sample showed a more narrow gelatinization temperature range.

10.4 RETROGRADATION OF STARCH

The changes that occur in gelatinized starch, from initially an amorphous state to a more ordered or crystalline state, are referred to as retrogradation. These changes occur because gelatinized starch is not in thermodynamic equilibrium. The rheological properties will change, as evidenced by an increase in firmness or rigidity.

Loss of water-holding capacity and restoration of crystallinity will also become evident and increase on aging. These processes exert a major and usually not acceptable influence on the texture of foods rich in starch. Starch retrogradation is the main factor in the staling of bread and other baked products [132–135], although other factors are also involved [136].

Because the processes of recrystallization and increased firmness are both referred to as retrogradation and different techniques are used to measure them, the evaluation of retrogradation becomes complicated. Different techniques are not necessarily measuring the same process. The kinetics of retrogradation has been studied to elucidate the molecular mechanism behind the phenomenon but is still not completely known [132,133,135–139]. Retrogradation would not take place without a certain minimum amount of water, and the water content together with the storage temperatures are very important because they control the rate and the extent of retrogradation. Many substances can interfere with the retrogradation process. Most important among them are lipids and surfactants. The retrogradation tendency of starches of various botanical origins varies greatly and does not seem to depend simply on the amylose-to-amylopectin ratios of the starches.

10.4.1 METHODS FOR ESTIMATING RETROGRADATION AND THE FEATURES MEASURED

The most common methods for measuring retrogradation (i.e., rate and extent of recrystallization on aging) are x-ray diffraction analysis [133,140,141], thermal methods such as DSC [91,134,142–145], and rheological techniques [138,146–148]. Because the retrogradation is to a large extent a recrystallization process, it can be followed by changes in x-ray diffraction patterns. In cereal starches, the A-pattern is lost during gelatinization and only the V-pattern is obtained due to the formation of an amylose–lipid complex. On aging, the B-pattern will develop, superimposed on the V-pattern [140]. The intensity of the B-pattern increases with time. X-ray diffraction analysis gives, therefore, both the type and degree of crystallinity.
Thermal methods (e.g., DSC) are well suited for following the rate and extent of retrogradation as the starch molecules progressively reassociate on aging. Aged gels and stale bread show a characteristic melting endotherm around 55 to 60°C, which is absent in fresh gels and breads immediately after gelatinization. This transition enthalpy increases progressively in magnitude with storage time until a certain limit is reached and remains constant on further storage. The calorimetry provides, therefore, a means to follow the formation of recrystallized starch gels through the melting endotherm of the B-crystals. The endotherm measured is the melting of recrystallized amylopectin [91]. Rheological techniques, especially fundamental viscoelastic measurements, are also well suited to monitoring gel firmness (rigidity) on aging. Other methods, such as enzymatic digestion [149], quantitative centrifugation [150], Raman spectrometry [137], and the NMR technique [151] have been used to evaluate the retrogradation process.

10.4.2 Components of Starch

It was first suggested by Schoch and French [132] that the staling of bread essentially involves the retrogradation of the amylopectin but not the amylose fraction. Since then, many investigations have been carried out to determine the respective roles of amylopectin and amylose and their combined effects in the retrogradation of starch gels and staling of baked products. The composite nature of starch gels, in which swollen gelatinized starch granules are embedded in an interpenetrating amylose–gel matrix, to a large extent determines the roles of both amylose and amylopectin [152–155].

When gels that are made of amylose or amylopectin (without granules) are compared to starch gels, some important features emerge that explain the roles of the two starch polymers in retrogradation. Early x-ray diffraction studies on aged starch gels showed that the B-type diffraction pattern developed slowly [140]. Amylose gels in storage and amylose precipitated from aqueous solution give weak x-ray diffraction patterns of the B-type [156]. Amylopectin gels also show the characteristic B-type pattern upon storage [157,158]. Both amylose and amylopectin gels, then, show the B-pattern upon storage. Sarko and Wu [40] proposed that the retrogradation is due to crosslinking of chains by double-helical gel junction zones. One possible mechanism involved in the gelling of amylose is phase separation into polymer-rich and polymer-deficient regions [146,147]. Crystallinity, as detected by x-ray diffraction, is a slower process than gel network formation (i.e., phase separation) and was proposed to occur in the polymer-rich regions of the gel [146,147]. For both amylose and the starch gel, the initial development of crystallinity was found to occur at similar rates. The crystallization of amylose reached a limit after 2 days, whereas the crystallinity of the starch gel continued to increase [147]. The amylopectin gels increase slowly in crystallinity with time.
and approach a limiting value after 30 to 40 days [158]. It was found that about 70% of the crystallinity of fully retrograded starch gels was lost after heating to 90°C, whereas the crystallinity of the amyllose gel was reduced by only 25% [146]. The crystallinity of amylopectin gels is fully reversible by heating [157]. The residual crystallinity of starch gels after heating is therefore solely due to the amyllose fraction. Isolated gelatinized starch granules that are mostly made of amylopectin and washed free from all exuded amyllose give no x-ray diffraction pattern immediately after cooling. After 2 weeks of storage, the B-type pattern is obtained, which completely disappears upon heating to 70°C [146].

Differential scanning calorimetry studies on retrogradation also suggest that long-term changes are due to the amylopectin fraction [91,142,146]. Aged bread, starch, and amylopectin gels show a melting endotherm that slowly increases with time, whereas no melting endotherm is obtained for amyllose gels in the temperature interval of 10 to 130°C. The crystallinity of the amyllose fraction can be seen as an endothermic peak at 145 to 153°C [142,159], a temperature rarely reached in connection with starch-based foods. The melting endotherm of starch gels and stale breads is completely reversible; no endotherm is obtained immediately after the heating of an aged starch gel. In a DSC study on amyllose chain association in lipid-depleted starches and amyllose, an exothermic peak appeared on cooling immediately after the samples had been heated to 180°C [159]. This shows that the amyllose reassociates very quickly, as waxy maize starch or amylopectins did not show this exothermic peak. The different recrystallization rates of amyllose and amylopectin have been confirmed by microcalorimetry, where the exothermic heat evolved during crystallization is measured [159a].

10.4.3 Interactions of Amylopectin and Amylose

In a study in which retrogradation of gels from nongranular mixtures with different amyllose/amylopectin ratios were studied, synergistic interactions were seen between amylopectin and amyllose at a high amyllose content [160]. Because the melting endotherm, as measured by the DSC method, has been attributed to the recrystallization of the amylopectin fraction, one could expect that the melting endotherm is proportional to the amount of amylopectin. Gudmundsson and Eliasson [160], however, found unexpectedly high values for the melting enthalpy of gels with very high amyllose content (75 to 90%). The possibility of limited cocrystallization has been proposed in relation to retrogradation [161]. Such cocrystallization could be promoted when amyllose is found in high amounts. Schierbaum et al. [162] have found that linear segments of amylopectin and amyllose, or limit dextrins of certain critical lengths, can interact in solution. Similar findings were reported by Seivert and Würsch [159] in a study of the chain association of amyllose and the effect of amylopectin on that process in mixtures with different ratios of amyllose to
amylopectin. They found that an increasing amount of amylopectin restricted the chain association of amylose, and the authors attributed this finding to either dilution or steric hindrance effects; however, the amylose and amylopectin in aqueous solution have been shown to be immiscible at moderate concentrations, and that encourages phase separation of the polymers [56]. Under most circumstances, the interactions of amylose and amylopectin should be limited in normal starch gels, as amylose is preferably leached out of the granules, whereas the amylopectin is mainly retained within the granules.

10.4.4 Storage Temperature and Water Content

Retrogradation is greatly affected by storage temperature. Storage of starch gels with 45 to 50% water content at low temperatures but above the glass transition temperature \( T_g = -5.0°C \) increases the retrogradation compared to storage at room temperature, especially during the first days of storage. Storage at freezing temperatures below the \( T_g \) virtually inhibits recrystallization [91,134]. Higher temperatures (above 32 to 40°C) effectively reduce retrogradation [134]. The Avrami equation has been frequently used to account for the kinetics of the recrystallization process at different temperatures and water contents [134,135,137]; however, the analysis of retrogradation kinetics according to the Avrami equation requires thermodynamic equilibrium conditions, but that is not the case here and the method therefore has limited applicability. Retrogradation is a nonequilibrium recrystallization process, as indicated by the fact that at low temperatures (4 to 5°C) the crystallites formed are less nearly perfect (i.e., they have lower melting temperature \( T_c \)) than crystallites formed at higher storage temperatures [163,164]. A three-step mechanism of initial nucleation (junction point of two or more starch molecules) followed by crystal growth and propagation and then crystal perfection has been proposed [6].

Crystallization that follows such a mechanism is nucleation controlled (i.e., the nucleation has to take place before the propagation can begin). Within the range \( T_g \) to \( T_c \) (e.g., –5.0 to 60°C for a gel with 50% water), both nucleation and propagation exhibit an exponential dependence on temperature, such that nucleation rate increases with decreasing temperature, down to the \( T_g \), while the propagation rate increases with increasing temperature, up to the \( T_c \) [6]. This explains why crystallization occurs at low temperatures but only to a limited degree at elevated temperatures (>30°C), because nucleation formation is then retarded. For longer storage periods, the retrogradation should be maximal at a temperature about midway between \( T_g \) and \( T_c \), as both nucleation and propagation then take place at moderate rates. Both normal and waxy starches seem to follow this mechanism; the rate of retrogradation was found to increase during a 48-hour period with decreasing temperature in the interval of 1 to 25°C [165]. Amylose gels stored at 6°C did not develop a staling endotherm during 48 hours of storage [165], indicating that crystallinity melted.
below 100°C is due to amylopectin. Results from NMR studies on the temperature dependence of retrogradation are consistent with these findings [151,166].

Recrystallization of amylopectin is very sensitive to the water content in starch gels. A starch content in the range of 10 to 80% is necessary for the development of the DSC endotherm [137]. The maximum crystallization has been measured at around 50% starch with DSC as well as with NMR [137,143,145,151].

In contrast to a native starch suspensions, the gelatinized starch gel is completely amorphous and its water is uniformly distributed. The recrystallization process depends on the temperature difference between the storage temperature and the $T_g$ of the amorphous gel, as the mobility of the chains determines their association rate. Because water is a plasticizer, it controls the $T_g$ of the amorphous gel. At a very low water content, the $T_g$ is above room temperature, and the amorphous gel is in a highly viscous glassy state that effectively hinders molecular mobility. Recrystallization increases with increasing water content until 45 to 50% water content is reached. Progressively more effective plasticization (increased molecular mobility) is obtained, and finally $T_g$ is depressed below room temperature. Recrystallization then decreases with a further increase in water content up to 90%, apparently due to dilution of the crystallizable component in the plasticized amorphous matrix [6].

Due to their antiplasticizing effect, solutes (e.g., sugars) affect the retrogradation of starch gels compared to water alone [6]. They reduce the mobility of the chains in the amorphous matrix by increasing the $T_g$. As a consequence, the rate of propagation can decline, decreasing the extent of retrogradation.

10.4.5 Botanical Source

The botanical source is of great importance for the retrogradation of starch gels [22,167–173]. This is true not only for starches with very different amyllose content, but also for starches with similar amyllose contents. Some of the differences among, for example, cereal starches can be attributed to differences in the amyllose/amylopectin ratio and lipid contents; however, these factors account for only some of the differences. Structural differences found in the amylopectin molecule can explain some of the differences in the rate and extent of recrystallization.

Some studies indicate that the rate, and sometimes the extent, of retrogradation increases with increasing amounts of amyllose. Although the amylopectin is considered responsible for the long-term retrogradation, some waxy starch types are reported to retrograde slowly, but pea and potato starches with high amyllose contents retrograde to a greater extent [151,174,175]. It is possible that the initial rate of retrogradation could be accelerated because of synergistic interactions between amylopectin and amyllose, as discussed earlier. Other studies have failed to show this relation of
amylose content to retrogradation [91,145,160]. For DSC measurements, in particular, the extent of retrogradation should be greater for waxy starches because the DSC measures the recrystallization of amylopectin [91,142,160]. It is thus safe to say that the relation between the amylose/amylopectin ratio and retrogradation is not straightforward, as other factors are involved.

If retrogradation is to take place, the chains must first aggregate, but a minimum requirement for the aggregation of chains to occur has been shown to be 8 to 10 glucose units [47,176]. The short chains of amylopectin have been shown to be responsible for crystallization in the amylopectin molecule [177], and chains with less than 15 glucose units do not take part in the crystallization [158,177]. If the external chains of the amylopectin molecule are removed by β-amylolysis, no retrogradation at all occurs [178a,b]. Amylopectin from potato, tapioca, and kuzu starches, which are B-starches, retrograde to a different extent, which has been related to differences in average chain length [178]. Amylopectin from cereals has also been shown to retrograde to a lesser extent than pea, potato, and canna amylopectin, which has been attributed to shorter average chain length in the cereal amylopectin [167,173]. The length of the external chains in the amylopectin molecule is also of relevance [178a,b].

The transition temperature, $T_c$, at which the melting of the recrystallized starch occurs is nearly the same for all the cereal starches (with the exception of amylomaize), despite differences in gelatinization temperatures of up to 24°C [171]. The gelatinized starches are fully hydrated, and the recrystallized (retrograded) starch melts according to its melting temperature at the existing water content. The similar $T_c$ values of cereal starches indicate that their crystallites have similar stability and are therefore of similar short chain lengths. A higher melting temperature $T_c$ has been reported for B-starches with longer short chains [173]. The structural differences in cereal amylopectins related to retrogradation can be related to differences in the amorphous regions or differences in the ratio of short chains to long chains and the ratio of A chains to B chains. A greater amount of short chains over 15 glucose units and an increased ratio of A chains to B chains probably promote retrogradation. It has also been reported that very short chains (6 to 9 glucose units) can inhibit or retard retrogradation of starch gels [179,180].

10.5 RHEOLOGICAL BEHAVIOR OF THE STARCH GEL

The events occurring during gelatinization of a starch suspension, especially the swelling and leaking of amylose/amylopectin, will dramatically change the rheological properties of the starch suspension. The subsequent retrogradation will then further modify the rheological properties. Perhaps the most common method for studying starch properties is to study the changes in viscosity during a programmed heating–cooking–cooling cycle. The measurements are carried
out in instruments such as the Brabender Amylo/Viscograph. Here the heating rate is usually 1.5°C/min, and the heating is performed during stirring. After a holding period (10 to 30 min) typically at 95°C, cooling at 1.5°C/min is performed. The viscosity (measured in Brabender units [BU]) is recorded as a function of temperature; a typical viscogram is illustrated in Figure 10.6. The Rapid Visco Analyzer (RVA) is emerging as a more popular alternative because of the need for smaller sample amounts and shorter analyzing times [180a,b].

For the application of starch in a food process, the rheological behavior during different temperature regimens is of interest, and some of these aspects are examined in a viscogram. The increase in viscosity during heating is of interest in relation to the equipment used. Changes in viscosity during a cooking period give indications of the stability, and the changes occurring during cooling might show the consistency of the product when consumed; however, products are stored either at room temperature or in refrigerators or freezers, and during storage changes in rheological properties occur. If the product is heated again before consumption, new changes in rheological properties can be expected. Moreover, process conditions might be very different from the conditions in an instrument such as the Amylograph. Thus, a broad range of different rheological measurements might be required to fully characterize a starch in relation to its utilization in a certain food product. In the following text, results obtained by fundamental rheological measurements are reported. The starch gel is characterized with certain moduli, such as shear...
modulus ($G$), storage modulus ($G'$), loss modulus ($G''$), or complex modulus ($G^*$). The phase angle ($\delta$) is used to indicate the degree of elasticity.

To understand the rheological behavior of starch it is helpful to regard the gelatinized starch suspension as a composite material composed of a dispersed phase (starch granules) in a continuous polymer solution (amylose–amylopectin) [152,153,157]. The rheological properties of such a system depend on properties of the components themselves as well as their ratio and interactions between them [93]. In the following sections, the rheological properties of the components (i.e., amylose and amylopectin) alone or in mixtures are described. The rheological properties of starch gels, during gelatinization, during storage, and in relation to freeze–thaw stability are then described, as well as how these properties can be modified by the processing conditions.

### 10.5.1 Amylose Gelation and Amylose Gels

Amylose solutions are unstable at room temperature. They will show turbidity and eventually form a precipitate (from dilute solutions) or a gel (from concentrated solutions). With time, syneresis often occurs (i.e., the formation of a liquid film on top of the gel). To explain the mechanism for this behavior, the gelling of amylose can be compared with the gelling of other linear polymers.

The first step in the gelation of amylose is the formation of a network. For quenching of amylose solutions (i.e., rapid cooling from a hot solution), it has been shown that for concentrations above a certain value ($C^*$, the overlapping concentration), a gel is formed, whereas for concentrations below $C^*$ a precipitate is formed [147]. The problem is how to determine $C^*$, and it has been suggested that the gel formation concentration ($C_0$) is somewhat below $C^*$ [181]. Amylose was shown to form a gel at a concentration greater than 1.5\% (w/w), and above this concentration $G$ was proportional to $C^*$ [115].

Turbidity is developed in an amylose solution after a certain time, known as the cloud time [181]. The cloud time increases with increasing temperature and is almost infinite at 42°C [181]. An increase in $G'$ is observed during the same time as the clouding (Figure 10.7) [181]. The increase in turbidity might precede the increase in $G'$ or lag behind, depending on the amylose chain length [182]. The development of turbidity indicates aggregation; a phase separation into a polymer-rich phase and a polymer-deficient phase occurs. It has been suggested that gelation occurs due to entanglement in the polymer-rich phase [115]. The critical concentration for gelation, $C_o$, of amylose has been determined to be approximately 0.9 to 1\%, and this value was found to be independent of chain length, at least in monodisperse amylose samples [181,183].

After the network is established, its strength improves with time. The changes with time are attributed to crystallization in the polymer-rich phase [147], and the B-pattern can be measured by x-ray diffraction [183]; however,
the crystallinity increases at a slower rate than turbidity or gel strength [184].

Double helices are found in precipitates as well as in gels [183], but not all
of these double helices form crystalline domains. Around 18 to 33% of the
polysaccharide forms an amorphous fraction [185]. It has been suggested that
these double helices could constitute a gel formation mechanism though inter-
chain double helix formation, without the prerequisite of phase separation [183].

Amylose gels are thermally very stable; they do not melt even after being
heated to 120°C in an autoclave [115]. The peak temperatures measured by
DSC range from 117 to 125°C for amylose gels at concentrations of 2.73 to
7.94% [185].

10.5.2 AMYLOPECTIN GELATION AND AMYLOPECTIN GELS

Amylopectin solutions are often regarded as stable, but under the proper
conditions (usually low temperature and high concentration) gelation occurs.
An increase in turbidity was observed with decreasing temperature from 1 to
20°C for amylopectin [158]. Gels were observed to form for solutions with
concentrations above 10% at temperatures below 5°C. For amylopectin from
potato and tapioca, translucent gels were formed, whereas turbid gels were
formed from cereal and fava bean amylopectin. The gel formation rate was
very slow, and a constant value of the shear modulus was not obtained even
after 30 to 40 days (see Figure 10.8). The rate of gelation differed for different
sources of amylopectin and was very high for pea and very low for maize
[173]. When the modulus (G') was plotted against concentration, a linear
relationship was found, compared with the dependence on C^3 for amylose
Treatment of amylopectin with β-amylase to produce β-limit dextrins destroyed the gel formation ability [158]. The outer branches of the clusters in the amylopectin molecule thus seem to be necessary for gel formation. The amylopectin gels are thermoreversible; they melt when heated to 40 to 60°C [91,158]. A stable enthalpy value has been obtained after about the same time as a stable modulus.

10.5.3 Amylose and Amylopectin

The incompatibility between amylose and amylopectin, discussed previously, certainly influences the type of gel formed. Measurements of the elastic modulus ($E$) for amylose–amylopectin gels after different aging times have shown a complicated dependence on the amylopectin concentration [57]. Gels formed with amylose and amylopectin at different amylose/amylopectin ratios ($r$) were studied at a total polysaccharide concentration of 8% [186]. Gels formed at $r > 0.25$ (corresponding to 1.6% amylose) for these mixtures. Below $r = 0.43$ (corresponding to 2.4% amylose), the mixed gel behaved like an amylopectin gel, and above $r = 0.43$ it behaved like an amylose gel. In both studies, it was suggested that the structure of the gel was a continuous phase with a dispersed phase, and at a certain concentration (e.g., $r = 0.43$ [186]) an inversion point can be obtained. Below this value, amylopectin is continuous, and above it amylose is. A local cocrystallization was also suggested to occur at the interface of microdomains. Different properties could thus be expected to be obtained for the starch gel depending on the amount and type of material solubilized during gelatinization. High soluble amylose levels and swelling powers have been found to increase elasticity, whereas high levels of soluble amylopectin are detrimental to gel formation and reduce elasticity [187].

FIGURE 10.8 Changes in shear modulus with time for potato starch amylopectin. (Data from Kalichevsky et al. [173].)
10.5.4 Starch Granules

A gelatinized starch suspension forms a more rigid gel than can be expected from the rigidity of an amylose gel formed from the amount of amylose leached [146,157]. Moreover, the solution centrifuged from such a gelatinized starch suspension shows very low viscosity [188]. The granules thus are important for the rheological properties of the starch gel. Starch granules remain more or less fragmented after most heat treatments [59,60,154,189]. The function of the granules could be to act as filters in the amylose–amylopectin matrix [153]. The starch granules influence the rheological properties of the starch gel due to their phase volume and their deformability, but also due to adhesion between the filler phase and the matrix. These parameters usually influence each other, so it is difficult to isolate the influence of one single parameter. It has been suggested that in dilute suspensions viscosity is governed by the volume fraction of swollen granules, whereas in concentrated systems it is governed by particle rigidity [155]. Dynamic mechanical thermal analysis (DMTA) on wheat starch preparations at intermediate moisture (25 to 60%, w/w) indicated an increase in $G'$ due to swelling, followed by a decrease due to melting and softening [189a].

10.5.4.1 Phase Volume

The weight percent of starch to reach close-packing differs among starches; for example, it is 2.8% for corn and 0.25% for potato [188]. It is thus evident that in most applications the starch granules will be close-packed in the gel. The shape and size distribution of the granules will affect the packing behavior and influence the level of starch required for close-packing. When the granules are close-packed, less soluble amylose will be present.

The viscosity ($\eta$) of a starch suspension — expressed as $\eta/CQ$, where $C$ is the concentration (g dry starch per g suspension) and $Q$ is a quantity describing the swelling of the particle in dilute suspension (g swollen starch per g dry starch) — has been plotted against $CQ$ [154]. The values obtained for different starches and different cooking conditions could be superimposed, but the fit was not always perfect; however, the results still illustrate the importance of the phase volume ($\varphi$) of the swollen granules.

Attempts have been made to account for the solubilization of material from the starch granules when $\varphi$ is calculated [28]. The $\varphi$ for a single starch is very much related to the cooking conditions, and a reason for the different values in viscosity obtained from different methods is that different $\varphi$ values are obtained depending on the heat regimens and mechanical treatments used.

10.5.4.2 Deformability

Because the starch granules are close packed in most food gels, their deformability has to be taken into consideration when analyzing the rheological
behavior. The resistance against deformation has been measured for individual gelatinized starch granules, and considerable variation was found between individual granules [190]. The variability was not related to granule size.

It is possible to change the deformability of the starch granules, and softening during gelatinization is one obvious example [153]. For a heated maize starch suspension two types of flow behavior have been observed: shear thinning in combination with a Newtonian region or a dilatant behavior [191]. The dilatant flow was suggested to occur when the granules are close packed but too rigid to deform. A shear-thinning behavior can be imposed either by an increase in temperature (to make the granules softer) or an increase in concentration (to increase the stresses applied). For the shear-thinning behavior to occur, the granules must have lost their birefringence.

The rigidity of starch granules will depend on the degree of gelatinization. When concentrated wheat starch suspensions were heated to different temperatures, corresponding to the double DSC endotherm obtained at limited water levels, an increase in relaxation modulus ($G$) was first obtained (i.e., during the first peak in the thermogram). With further heating (i.e., when the second peak in the thermogram was reached), $G$ decreased [153]. This was interpreted as being due to the starch granules becoming softer when more of their crystallinity was melted. Similar results were obtained when pea starch granules were added to an amylose solution at $\varphi = 0.8$ [157]. The reinforcement of gel rigidity decreased when the temperature to which the granules had been heated increased.

When gelatinized starch was treated with enzymes (salivary amylase), the resistance against deformation decreased considerably, although the diameter did not change [190]. At the same time, the shear stress of the gelatinized starch suspension decreased. The reason was not that the phase volume changed but that the deformability of the individual granules in the dispersed phase decreased. With time, crystallinity develops in starch gels, and the B-pattern emerges [146]. The development of crystalline domains will cause the deformability of the granules to decrease, and more rigid gels are thus obtained.

It is not always possible to separate the effects of phase volume and deformability. The contribution of phase volume and deformability can also change with concentration. For several starches, a crossover in plots of viscosity vs. concentration has been observed [155]. At low concentrations, the high-swelling starches have a higher viscosity than low-swelling starches, and at high concentrations the reverse is true.

10.5.4.3 Adhesion

The rheological behavior of a gelatinized starch suspension might also depend on adhesion between the dispersed phase and the matrix. The influence of lipids on the rheological properties of a starch suspension has been attributed partly to changes in adhesion between the filler and matrix [192]. Interactions
between starch and other polysaccharides have also been interpreted in terms of changes in adhesion [193].

10.5.5 Starch Gels

The rheological properties of a starch suspension will change during at least three processes of relevance to food processing: gelatinization, retrogradation, and during freezing and thawing; thus, it is necessary to be able to measure the rheological properties of starch gels during and after these processes have taken place. As is described in this section, the rheological properties are influenced by starch source, starch concentration, temperature, heating rate, and mechanical treatment. Storage temperatures also have an influence. Furthermore, the addition or presence of other components (e.g., lipids, proteins, other polysaccharides, sugars, salts) influences the rheological properties.

10.5.5.1 Gelatinization

To follow the changes in rheological properties during the gelatinization of starch, a viscoagram (Figure 10.6) can be recorded. It is apparent that an increase in viscosity is not measured until swelling and leaking have proceeded to some extent. The increase in viscosity is due to the swelling of starch granules and can be rationalized by the increase in phase volume, as has been investigated in, for example, maize starch [194]. The situation is more complicated, however, than the mere phase volume. The shape of the granules influences their packing behavior, and the deformability of the granules then comes into play. The viscoagram reveals another effect that contributes to the development of a peak in viscosity — shearing causes fragmentation of the starch granules and perhaps even complete dissolution of the granules.

The mechanical degradation influences the leaking of amylose and amyllopectin from the granules. As already discussed, amylose and some amyllopectin leach out from the granules during heating without any stirring. With shearing, this leaking is enhanced, and the composition of the leached material is certainly affected. This, in turn, affects the properties of the continuous phase, due both to concentration effects and the nature of the material leached.

It is thus easily understood that the rheological properties of a starch gel or paste will very much depend on the preparation procedure (i.e., time, temperature, and mechanical treatment). Moreover, each type of starch will react in its own way to a given set of conditions. It is also evident that native starch, as has been described so far, is easily destroyed during cooking. The reason for using modified starches, or starch derivatives, is easily understood.

Starch gels characterized at room temperature after gelatinization showed a shear-thinning behavior [28,111,194–196] (see Figure 10.9a). Dynamic measurements show that the frequency behavior of the viscosity and storage modulus is typical for a weak gel [197–199] (Figure 10.9b).
10.5.5.1.1 Starch Source

When different starches are compared at the same concentrations and after the same preparation procedures, the rheological properties are very much related to the source of the starch. Measurements of the shear modulus have given the following order for starches: potato < wheat < maize < pea [167]. A similar ranking (tapioca < potato < wheat < maize) was obtained when the $G'$ values were compared, although at a lower concentration wheat starch was found to give lower values than potato starch [200]. The viscosity of oat starches was found to be higher than for maize and wheat [111], whereas the reverse was found for $G'$ [168]. At certain concentrations, however, a crossover might occur, and the ranking at a low concentration might be reversed at a higher concentration [155].

FIGURE 10.9 (a) Apparent viscosity vs. shear rate for a heated wheat starch dispersion (9%). (Data from Ellis et al. [114].) (b) Mechanical spectra (▲, storage modulus $G'$; △, loss modulus $G''$; ○, phase angle δ) for a wheat starch gel (6.6%). (Data from Lindahl and Eliasson [199].)
Differences are found even among different varieties of the same species. Differences in viscosity and the dynamic behavior of wheat starches have been attributed to the swelling behavior and number fraction of large granules [195,197]. Large differences have also been observed for rye starches [22] and for waxy starches (rice, barley, and maize) [171]. In the case of barley and maize starches, differences can be attributed to the amylose content [172,201]. The rheological properties were studied for mixtures of waxy and nonwaxy wheat starches with amylose contents ranging from 5 to 25% [200a]. With lower amylose content in the gel, $G'$ decreased and the frequency dependence in dynamic viscoelastic measurements increased; that is, a more liquid-like behavior was recorded. The gels with more amylopectin showed a higher rate constant of starch retrogradation, measured as the increase in $G'$ with storage time.

The differences in pasting behavior between different starches are related to the fine structure of amylopectin, such as amylopectin branch chain-length distributions [201a]. The relationships between molecular structures and pasting properties for wheat starch have been studied using the RVA [201b]. A strong positive correlation was found between viscosity maximum in the RVA and degree of polymerization of both amylose and amylopectin. Also, the amount of extra-long chains in amylopectin was suggested to influence the viscosity in the RVA. For oat starches, it was found that $G'$ increased and the phase angle decreased with the lipid content of the starch [168].

### 10.5.5.1.2 Starch Concentration

For a viscosity to be observed, a certain concentration has to be reached: the close packing concentration [188,195]. Above this concentration, the viscosity as well as storage modulus and complex modulus increase with concentration. This has been observed for several starches, including wheat [110,195,197], oat [111], potato [189], and maize [167,202]. When potato, tapioca, maize, and wheat starches were investigated over a wide concentration interval (0 to 20%), it was found that $G'$ increased more rapidly at concentrations above a certain value typical for the starch [200]. The phase angle has been observed to decrease with concentration and then level off at a rather low value, the plateau value depending on the starch source [155,200]. The relationship between rheological properties and concentration is affected by the cooking procedure. For low-sheared samples, the phase angle decreases with concentration, whereas for sheared pastes the phase angle was found to increase as well as decrease with concentration, depending on the heat treatment [118]. The complex modulus was found to increase with concentration.

### 10.5.5.1.3 Temperature

Depending on the end temperature of the heating process, very different rheological properties during subsequent cooling were observed for oat and barley...
starches [59]. When the dispersions were heated at 95°C instead of 90°C, much lower $G'$ values and higher phase angles were observed during cooling. For maize starch, it has been observed that the same viscosity was obtained for an 8% suspension heated to 80°C as for a 12% suspension heated to 67°C [154]. For waxy maize starch, it was found that heating to 80°C instead of 90°C increased $G'$ and decreased the phase angle [201]. These results show that changes in the preparation procedure can be used instead of an increase in concentration to influence the rheological properties of a starch gel.

10.5.5.1.4 Heating Rate and Time
For a maize starch suspension, it was found that the heating time at a fixed temperature (65°C) influenced the apparent viscosity — longer heating times increased the viscosity [154]. For waxy maize starch gels, the reverse was obtained — the longer the heating times at a certain temperature (95°C), the lower the $G'$ for the gel at room temperature [201]. The discrepancy is probably related to the different temperatures: heating at 65°C could result in increased swelling, whereas heating at 95°C could result in increased fragmentation and dissolution. Rapid heating has been found to result in higher viscosity than slow heating for wheat and maize starches, at least during low shear conditions [110,196]. Wheat starch seems to be more sensitive to the heating rate than maize starch [114].

10.5.5.1.5 Mechanical Treatment
In general, decreased viscosities or moduli are observed with more intense or prolonged mechanical treatment. This has been observed for several starches, including wheat and maize [110,196]. To study the influence of the mechanical treatment, potato starch samples were transferred from the Brabender visco-graph to a rheometer and then characterized by oscillatory measurements [118]. A maximum in $G^*$ was obtained at about 65°C (i.e., at the same temperature as the peak viscosity in the visco-graph). Further treatment in the visco-graph caused a drastic decrease in $G^*$. A minimum plateau value was reached after holding the sample at 90°C for 10 minutes. At the same time, a maximum plateau value was obtained in the phase angle. When the samples were transferred at the temperature corresponding to peak viscosity, the changes in modulus due to the heat treatment in the rheometer were much smaller. The temperature did not at all affect the phase angle. For pastes prepared in the visco-graph, the samples up to 75°C were characterized by a moderately elastic response ($\delta = 10^\circ$) and a medium high shear resistance ($G^* = 100–200$ Pa). Further heating and shearing resulted in a less elastic behavior ($\delta = 30^\circ$) and a very low complex modulus (10 Pa). For extruded starch, the viscosity for the suspension decreased with increased mechanical treatment; that is, increasing the time for treatment and the rpm decreased the viscosity [203].
10.5.5.2 Retrogradation

The changes in properties of a starch gel with time, known as retrogradation, have been discussed in a previous section. Some of these changes occur during the cooling curve in the viscogram (i.e., the retrogradation due to the amylose). During the storage of a starch gel, the rigidity continues to increase with time. This increase is reversible to some extent, as the part related to the crystallization of amylopectin can be reversed by heating. As was the case with rheological properties obtained during gelatinization of starch, the retrogradation can also be influenced, both by the choice of starch and by the preparation procedure, although this approach has been investigated to a much lesser extent. Most of the information available is related to the staling of bread.

10.5.5.2.1 Starch Source and Concentration

After a week of storage (20°C), the shear modulus has been found to differ between starches in the following way: wheat < potato < maize < pea [167]. The rate of development of the shear modulus depends on the starch source and was found to increase in the following order for different amylopectins: wheat < barley < maize < canna = potato < pea [173]. For different starch gels (40% starch) stored at 20°C, the compression modulus ($E$) after 10 days increased in the following order: waxy rice < wheat < manioc < rice < pea < potato [204]. The change in $G$ with time depends on concentration, and $G$ is always higher at higher concentrations [167,204]. Moreover, at low concentrations, a plateau value was found after a short time, whereas for maize starch gels at concentrations of 30 and 40% $G$ was found to continue to increase even after several days.

10.5.5.2.2 Heating Rate and Temperature

For bread baked in a resistance oven (i.e., without the formation of crust), the firmness was higher for longer heating times [136]. The development of rigidity of a starch gel proceeds faster at lower temperatures, with the following ranking being found for wheat starches: $10^\circ$C > $15^\circ$C > $20^\circ$C > $30^\circ$C [148].

10.5.5.3 Freeze–Thaw Stability

For a starch gel that is frozen and eventually thawed, the rheological properties usually differ from the corresponding gel that has not been frozen. A very obvious effect of freezing and thawing is syneresis and the liquid-phase exudates present after thawing. The most important factor for determining the properties of a starch gel after freezing and thawing is the freezing rate (i.e., the size of the ice crystals formed). Certainly the conditions during thawing are also important, but the thawing rate is much more difficult to control. As discussed in the following text, the source of starch, the concentration, and the sample preparation procedure all exert an influence as well.
One method to characterize freeze–thaw stability is to measure the extent of syneresis [205,206]; however, the result is very much related to the experimental conditions (e.g., the centrifugal forces) [207]. As an alternative procedure for evaluating freeze–thaw stability, a rheological method has been developed [207] in which the starch gels are transferred to a rheometer after freezing and thawing, and small-amplitude oscillation experiments can be carried out at a fixed frequency during both heating and cooling. Together with the determination of freeze–thaw stability with respect to syneresis, some insights into the mechanisms can be obtained. No changes in $G^*$ with temperature and only a slight decrease in $\delta$ were found when hydroxypropyl potato starch gels were heated before freezing [207]. For freeze–thawed starch gels, it was found that $G^*$ increased with the number of freeze–thaw cycles until a plateau value was reached; at the same time, $\delta$ decreased. The freezing and thawing thus transformed the gel into a more elastic one, with an increased strength. Initially, when $G^*$ increased and $\delta$ decreased, a more perfect network was created; however, with each increasing freeze-thaw cycle, $\delta$ increased and $G^*$ was constant or decreased. This finding could be the result of disruptions in the network due to large ice crystals. When the gels were heated, $G^*$ began to decrease and $\delta$ increased. A peak value in $G^*$ and a minimum in $\delta$ were observed at the same freeze–thaw cycle as when syneresis was detected. This was suggested to be the effect of two processes: swelling of aggregated starch molecules (presumably amylopectin as well as amylose) and melting or dissolution (presumably amylopectin) of aggregated or crystallized molecules.

10.5.5.3.1 Starch Source and Concentration

Native starches show very poor freeze–thaw stability, and chemical modifications such as acetylation or hydroxypropylation are used to improve freeze–thaw stability [205–207]. Combinations of acetylation with crosslinking did not much improve the freeze–thaw stability, whereas a combination of crosslinking with hydroxypropylation had a detrimental effect [208]. Among the native starches, oat starch has been reported to show less syneresis than wheat starch [112]. A rather poor freeze–thaw stability was observed for pigeon pea starch — 30 to 50% syneresis, depending on the number of freeze–thaw cycles [113]. The freeze–thaw stability was improved, however, with defatting and even more so with heat–moisture treatment (30% water, 100°C, 16 hr). Defatting has also been found to improve the freeze–thaw stability for oat starch [112]. The rheological changes of starch pastes after freeze–thaw treatment depend strongly on concentration [209], and the rheological response connected with syneresis is delayed with regard to the number of freeze–thaw cycles when the concentration is increased.

10.5.5.3.2 Preparation Procedure

The influence of preparation procedure was studied by a viscochrograph [209]. Starch pastes (hydroxypropylated potato starch) were prepared by heating to
(1) peak viscosity, (2) half-breakdown, (3) 95°C for 15 minutes, or (4) the total cycle. The $G^*$ after preparation and at room temperature was highest for the paste prepared to peak viscosity and lowest for the paste that had gone through the total cycle. The $\delta$ values were the opposite. The rheological changes typical for freeze-thaw damage developed first in the paste that had been prepared at peak viscosity (after only one freeze-thaw cycle), then for the half-breakdown paste (two freeze-thaw cycles), holding period (six cycles), and total treatment (six cycles). This result indicates that complete swelling and leaking must occur during gelatinization; otherwise, these will occur during freeze-thaw treatment. Fragmentation of granules and their breakdown during the latter part of the viscogram should also occur; otherwise, these effects will be obtained during freezing due to ice-crystal growth.

10.6 MODIFICATION OF STARCH PROPERTIES

The behavior of starch described so far in this chapter can be modified in different ways. Modifications might occur because starch interacts with other components present in the food. The result might be detrimental for the quality or it might be necessary for obtaining the familiar structure of the food in question. In many applications, the properties of the native starch may not be the most appropriate, and then another source of starch might be used. An example is the use of certain genotypes with either an increased level of amylose (high-amylose varieties) or an increased level of amylopectin (waxy varieties). With increased knowledge about the biosynthesis of starch, new genotypes can be produced using modern gene techniques. Also, when the native starch does not have the desired characteristics, chemically modified starch is often used. In this section, these various types of modifications are described.

10.6.1 INTERACTION WITH OTHER COMPONENTS

In most applications of starch, other components are present, in addition to starch and water, and these other components have an effect on the starch behavior and starch properties. The effects observed may have different origins and can be due to soluble low-molecular-weight components (sugar, salt, acids) or to the presence of macromolecules, such as proteins and other polysaccharides. A special case is the interaction between starch and lipids because of the amylose-lipid complex. Recently, the formation of an amylopectin-lipid complex has also been verified in surface tension measurements, and by isothermal titration calorimetry [209a,b].

10.6.1.1 pH

The starch molecules themselves are uncharged in most starches. One exception is potato starch, which contains phosphate esters. It should therefore be expected that the pH (as well as ions) should influence the behavior of starch
only marginally, except in the case of potato starch. The gel strength (measured as $G^*$) of potato starch gels depends very much on pH and will be at a maximum at neutral pH values [210]. The phase angle has been shown to have a minimum at corresponding pH values. Other starches, such as tapioca, do not show this pH dependence for gel properties.

Retrogradation of wheat starch, as measured by DSC, is at a maximum at pH 5.6 and is lower at the pH values of 4.4, 7.8, or 9.4 [211]. The elastic modulus, on the other hand, has been shown to be lowest at pH 5.6 and highest at pH 9.4; however, the differences were small.

Extremely low pH values cause hydrolysis of starch and can be utilized as a method for the preparation of modified starch. The amorphous parts of the starch granules will be the first to be attacked, and the remaining crystalline parts are hydrolyzed at a much slower rate [212]. Extremely high pH values, on the other hand, cause cold gelatinization of starch. The starch granules swell at room temperature and amylose is solubilized in the presence of NaOH [213].

10.6.1.2 Salt

The rheological properties of potato starch are very sensitive to ionic strength, and $G^*$ is highest at low ionic strength [210]. No specific interactions with any ions have been detected, but all ions show the same effect when compared at the same ionic strength; however, starches other than potato are affected by the presence of ions. The influence of salt on the properties of starch (i.e., on the gelatinization temperature) depends on the type of salt as well as on the concentration. For NaCl, it was found that $T_o$, $T_m$, and $T_r$ first increased with the salt concentration and then decreased [80]. The gelatinization enthalpy showed a behavior similar to that for temperature — first an increase and then a decrease with concentration [214]. For potato and canna starches, a decrease in $T_o$ was measured, whereas for maize and rice starches (normal and waxy) first an increase and then a decrease were observed with increasing NaCl concentration [215]. Sodium sulfate caused a progressive increase in the gelatinization temperature of potato starch, from about 62°C to about 80°C, while sodium bromide decreased it to about 44°C [216]. At the same time, $\Delta H$ decreased. Salt (NaCl/starch ratio of 0.04:1) was found to increase $T_o$ and $T_m$ for rice starch at different water/starch ratios [217]. In the DSC thermogram at limited water levels, a shift in temperature occurred, but there were no changes in the presence of peaks [215]. Certain salts can gelatinize starch at room temperature; calcium chloride at a molarity around 3 $M$ is one example [80].

The retrogradation of wheat starch, measured as the melting endotherm of recrystallized amyllopectin by DSC, was found to decrease in the presence of NaCl [211]. The elastic modulus of the gels changed in a more complicated way with increasing NaCl concentration. It increased at low levels (0.44 and
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0.88%) and decreased at higher levels (2.21 and 4.43%). The anions increased the retrogradation rate in the order I⁻ < Br⁻ < Cl⁻ and F⁻, and the cations decreased in the order of K⁺ < Li⁺ < Na⁺ [218].

10.6.1.3 Sugars

The addition of sugars increases the gelatinization onset temperature [97, 214, 217, 219–221]. This has been known for a long time, and several explanations have been offered [219]. The presence of sugar means that there are fewer water molecules (i.e., the plasticizing effect of water decreases); consequently, the melting temperature of the starch crystallites increases [6]. When starch is gelatinized with limited amounts of water (i.e., when a double peak is present in the DSC endotherm), the addition of sugar causes the double endotherm to transform into a single peak [221]. Sugar will then decrease the gelatinization temperature range. This effect has been explained by the lower viscosity of a 1:1:1 starch–water–sugar mixture compared with a 1:1 starch–water mixture [221, 222]. At the same water activity (a_w) monosaccharides are less effective than disaccharides, which are less effective than trisaccharides [219, 223]. The volume of the solvent should also be taken into account, not only the volume of water [93a].

The swelling volume of wheat starch granules increases with sucrose concentrations up to a certain value (10% sucrose), then the swelling decreases [224]. The increase in granule diameter, observed in the microscope, is shifted to higher temperatures with increasing sucrose content [225]. In other words, at a certain temperature the diameter decreases with increasing sucrose concentration.

The rheological properties of a starch–water–sugar gel are different from the corresponding starch–water gel. The apparent viscosity of gelatinized starch suspensions was found to increase with sucrose concentrations up to a certain value, and then viscosity decreased [224]. Amylograph viscosity increases for wheat starch occur with increasing temperatures and increasing sucrose concentrations [225]. The influence on the viscosogram for corn starch is greater for disaccharides than for monosaccharides [226]. The influence on the swelling power of maize starch depends on the concentration; at the highest concentration, a decrease has been observed [226]. The effect is greatest for disaccharides compared with monosaccharides.

Sugars decrease the elastic modulus for stored starch gels [227], the effect being rather similar for sucrose, maltose, and glucose. The addition of sugars also has an influence on amylase extender starch gels and waxy starch gels. Added monosaccharides (ribose, xylose, and fructose) alter the crystallization of starch gels. Ribose and xylose were found to suppress the growth rate of amylopectin crystals. Fructose seems to influence both thermally reversible and irreversible crystalline components [228].

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10.6.1.4 Lipids

10.6.1.4.1 Amylose–Lipid Complex

It is well established that polar lipids (e.g., monoglycerides, fatty acids, and similar compounds) form a helical inclusion complex with the amylose molecule, between the hydrocarbon chain of the lipid and the helix of the amylose [156,229,230]. Two types of amylose–lipid complexes are known: type I, which is amorphous, and type II, which is crystalline [228a–c]. The crystalline inclusion complex gives rise to a so-called V-type x-ray diffraction pattern [140,231–233]. At high or intermediate water contents, DSC measurements indicate that these complexes melt at 100 to 120°C, with a higher transition temperature for type II complexes compared with type I complexes [232, 234–236]. The transition is reversible, as the complex reforms upon cooling.

The complex formation is dependent on the length of the carbon chains of the lipids and surfactants. Lipids and surfactants are required to have a minimum of 4 to 8 carbons in the chain [232,233], and optimal chain lengths are between 12 and 18 carbons [232,237,238]. Lipids and surfactants with fewer than 9 carbons in the chain give a complex of low stability (i.e., a low transition temperature) [232].

The effects of different lipid polar heads on the complex forming ability have been investigated by DSC [232,232a]. For a given chain length, the transition temperature is different for ionic and nonionic functional groups, but the specific nature of the groups has no significant effect. Differences are also found between saturated and unsaturated monoglycerides with regard to their abilities to form amylose inclusion complexes [239]. Saturated monoglycerides are by far more effective complexing agents in water because they are usually added in a suitable physical state (i.e., the lamellar liquid–crystalline phase) so the monomers can easily react with the amylose. Unsaturated monoglycerides, on the other hand, are ineffective when added as aqueous suspensions, as they form a cubic liquid–crystalline phase in which the monomers are much less available to react with amylose. If, however, the unsaturated monoglycerides are transformed into the lamellar liquid–crystalline phase, their ability to form an amylose–lipid complex is equal to saturated monoglycerides [239,240]. The unsaturated monoglycerides are either in a cis- or trans-form with respect to the configuration of the double bond. The trans- configuration has been shown to give more stable complexes, probably because the trans- configuration has a straight chain form [239,240]. Even long polyunsaturated fatty acids have the ability to form inclusion complexes [240a].

10.6.1.4.2 Gelatinization

The addition of polar lipids (e.g., monoglycerides) to starch-containing foods has an influence on the gelatinization properties [237,241]. The small amount of lipids present in the cereal starches affects their properties in a similar way.
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as added lipids. These lipids are either on the surface or inside the granules [242,243] and consist mainly of phospholipids or free fatty acids [244]. Ionic surfactants interact in a similar way with starch as monoacyl lipids by complex formation, but their being charged complicates their effects [236,245]. When amylose leaches out of the granules during gelatinization, the lipids, either native or added, form complexes with the exuded amylose, probably on the surface of the granules, and retard their swelling [246,247]; as a result, the gelatinization temperature is somewhat increased. Because the complex formation is an exothermic process that probably takes place during gelatinization, the enthalpy of the gelatinization measured via DSC could be observed to be lower than it really is [5,232a,235,236]. Certain surfactants (i.e., sodium dodecyl sulfate [SDS]) have been observed to decrease the gelatinization temperature of starches [245,247,248].

10.6.1.4.3 Rheological Properties

Monoacyl lipids and surfactants affect the rheological properties of the starches. They do so probably by changing the swelling and solubility of the starch granules. Previous studies on various lipids and surfactants have shown that they increase or decrease the starch viscosity, depending on the type of lipid and source of starch as well as experimental conditions [192,249–251]. The monoacyl lipids and ionic surfactants have a similar interaction mechanism, except that the ionic surfactants have an additional effect due to their charged nature [201,236,245]. Furthermore, when the lipids are introduced, whether they are heated with the starch suspension from the beginning or added at later stages (e.g., after the gelatinization), is a critical factor.

The volume occupied by the swollen granules is a dominant factor at low starch concentrations, as discussed in Section 10.5. In a system of low starch concentration, the effect of the lipids should be decreased viscosity because of the retarded swelling and solubility; however, a decrease in viscosity is only obtained as long as the lipids or surfactants can retard the swelling and solubility of the granules. At high temperatures (e.g., above 95°C), starches with added lipids or surfactants can have higher viscosity than starch pastes without added lipids [236]. At 95°C in systems with excess or intermediate water contents, many amylose–lipid complexes begin to melt [201,232,252], allowing for rearrangement of the complex [252], and the retardation effects disappear. At lower temperatures (e.g., 85°C), the lipids effectively retard the swelling and decrease the viscosity compared to starches without added lipids [236]. If the lipids or surfactants are added at later stages, when the starch has already been heated, smaller effects should be seen because the granules have swelled unrestricted.

Ionic surfactants have somewhat different effects, as they destabilize the starch granule; for example, increased swelling and solubility are observed when starch is heated in their presence [253]. This could be the result of an amylose extraction effect of the ionic surfactants, because of the charged and highly hydrophilic head group [109,192,245]. The viscosity should therefore
increase in their presence, and the charged head group further stabilizes the starch paste because of electrostatic repulsive forces. At 85°C, the viscosity increase is only due to the charged nature of the ionic surfactants, as their effect can be completely canceled by the addition of salt before heating. At 95°C, the addition of salt makes the ionic surfactants behave similarly to nonionic surfactants [236].

At high concentrations the situation is different. Granule-to-granule contact, and thus granule deformability, is the most important factor. The lipids or surfactants make the starch granules more rigid until a certain temperature is reached; therefore, starch pastes should become more viscous when lipids or surfactants are added, and gels should be firmer than starch pastes or gels without added lipids or surfactants. Lipids and surfactants that are added at later stages could be expected to affect only the continuous amylose phase. Other rheological properties (e.g., stickiness) are also affected when lipids are added because the complex on the surface of the starch granule reduces stickiness by decreasing the hydrogen bonds available.

10.6.1.4.4 Retrogradation

Monoglycerides and other related compounds are known to have an antistaling effect on bread and to extend its shelf-life [241,255,256]. It is believed that the retarding mechanism of lipids and surfactants on retrogradation is related to their ability to form complexes with the amylose fraction [140,241,255,256]. The effects of added lipids or surfactants on retrogradation can be measured with many different techniques, such as x-ray diffraction, DSC, or various rheological methods. The effect of lipids added to bread is an increase in V-type x-ray pattern compared to control bread without such an addition. The V-type pattern is virtually unchanged with time but is superimposed by a B-type x-ray diffraction pattern that increases on aging [140].

The effect of lipids or surfactants on retrogradation as observed with DSC is a decreased melting endotherm of recrystallized amylopectin, as well as an increased size of the endotherm associated with the amylose–lipid complex transition [143,144]. Rheological measurements (mostly firmness measurements) usually show that added lipids decrease the firmness of breads on aging compared to breads without added lipids [257]. Other rheological measurements have shown that surfactants can have varied effects on the rheological properties of starch gels depending on the type of surfactant [236,241]. The presence of emulsifiers in concentrated starch gels (40%, w/w) slows down the increase in firmness during aging, although the elasticity modulus initially is higher than for the control without emulsifiers [256a].

As discussed in Section 10.4, the amylopectin fraction is responsible for retrogradation [132], and the long-term effects associated with retrogradation are related to the amylopectin fraction. The obvious question then arises as to how lipids or surfactants can affect retrogradation, as they are believed to form complexes with the amylose molecule [140,255,258]. The possible alternatives
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are that either the lipids act through an amylose–lipid complex in various ways or the lipids interact directly with the amylopectin.

Three possibilities exist with regard to the amylose–lipid complex and retrogradation. First, it could be that the intact complex (i.e., as one entity) interferes with the crystallization of amylopectin and retards the retrogradation [259]. Second, the amylose–lipid complex could change or retard the water distribution and hence the retrogradation [260]. Third, a cocrystallization of amylose and amylopectin is possible to some extent, and substances that complex with amylose eliminate the contribution of amylose in the recrystallizing process [161]. On the other hand, the interaction of amylopectin and lipids means that lipids interact directly with the amylopectin fraction, at least to a small extent, and retard retrogradation through the formation of an amylopectin–lipid complex [6,160,171,172,236,261,262].

The possibility of retarding the retrogradation of some starches (maize, potato, and waxy maize) and nongranular amylopectin by adding an intact cetyltrimethylammonium bromide (CTAB)–amylose complex has been explored [259]. It was found that CTAB–amylose complexes added to a cooled gelatinized starch gel or to starch suspensions that were heated to temperatures below the transition temperature of the CTAB–amylose complex had little effect on retrogradation; however, starch gels or starch suspensions with added CTAB–amylose complexes that were heated to temperatures above the transition temperature (i.e., the complex melted) showed a decreased retrogradation. This mechanism is therefore unlikely.

Because lipids and some surfactants retard or delay gelatinization, probably by complexing with leached amylose on the surface of the starch granules, it is possible that lipids or surfactants also retard the retrogradation by a similar mechanism (i.e., by acting as a barrier against water transport) [260]. This mechanism could be involved in retarding the retrogradation of normal starches because it is known that the water content is very important for recrystallization; however, it does not explain the effect of lipids and surfactants on decreasing the retrogradation of waxy starches, unless they are able to interact with the surface molecules of the waxy starch granules. Furthermore, nongranular amylopectin gels have also been shown to decrease in retrogradation when surfactants and monoglycerides are added [160,261], which shows that the granule form is not a limiting factor. If lipids and surfactants are added after the gelatinization is concluded, such a barrier effect should not be obtained and the retrogradation should progress in a way similar to that for starch gels without additives; however, pregelatinization of the starches before the addition of a surfactant was found to decrease retrogradation compared to starch gels without such an addition [171,172]. This indicates that lipids or surfactants affect the retrogradation even after the starch suspensions are fully gelatinized. This alternative, therefore, has only limited value as an explanation of the effects of lipids or surfactants on retrogradation.

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Russell [161] has suggested that a possible cocrystallization of amylose and amylopectin contributes to retrogradation, and the role of lipids and surfactants could be to eliminate the participation of amylose in that process. As before, this mechanism does not explain the effects of lipids on waxy starches and nongranular amylopectin gels. It could possibly assist in delaying the retrogradation of the normal starch gels, but according to Gudmundsson [160] the amylose content of amylose–amylopectin mixtures has to be greater than 50% before it has any effect on the retrogradation of the amylopectin fraction. This alternative can therefore account for only a small part of the retarding effects of lipids and surfactants. Other explanations involving the amylose–lipid complex are possible but cannot account for the effect of lipids and surfactants on decreasing the retrogradation of waxy starches and non-granular amylopectin gels.

10.6.1.4.5 Amylopectin–Lipid Interactions
Because the formation of the amylose–lipid complex does not explain the effects of lipids and surfactants on retrogradation, another mechanism has been proposed. Retrogradation of waxy starch and nongranular amylopectin gels is greatly affected by added lipids and surfactants, so direct interaction between the amylopectin molecule and lipids or surfactants through complex formation has been suggested [6,160,171,172,236,261,262]. Amylopectin in solution does not precipitate when monostearin is added [237], and no DSC endotherm for a complex transition is seen when amylopectin is heated and cooled in the presence of lysolecithin or for waxy starches with added lipids [201,236,258,261]. These findings have been taken as evidence that interactions between amylopectin and lipids are negligible; however, lipids have been shown to decrease the melting endotherm even for waxy starches and nongranular amylopectin [236], an effect that cannot be explained by the formation of an amylose–lipid complex.

It is possible for the outer branches of the amylopectin to take part in forming a complex between amylopectin and lipids [236,261,262]. The outer branches of the amylopectin molecule have the shortest average chain length [26], and many of them do not reach the required minimum for a complex or a double helix formation to take place [47,177,233,263]. Although the lipid binding to amylose has been shown to be cooperative, the binding to amylopectin was found to be of Langmuir type [209a,b]. The lipid molecules will thus bind to equivalent binding sites in the amylopectin molecule, and the binding to these sites is not influenced by the fraction of bound molecules. As the complex formation between amylopectin and lipids or surfactants is a noncooperative interaction, it is not possible to observe the process as an endothermic transition in the DSC [236]. The melting of the complex would spread over a large temperature interval and the expected transition enthalpy is small, making it difficult to separate such a peak from fluctuations in the baseline. More direct
evidence on amylopectin–lipid/surfactant interactions has been reported by Slade and Levine [6], who obtained a transition endotherm with DSC for a complex between waxy maize starch and sodium stearoyl lactylate (SSL) at 70°C at a very low water content (10%). The authors suggested that such a complex is so unstable that it is only observed at very low water contents due to the plasticizing effects of water on the complex. Gudmundsson [160], however, obtained a complex transition endotherm for nongranular amylopectin gel with added surfactants (CTAB and SDS) and monoglycerides at around 110°C and at an intermediate water content (50%, w/w). It is possible that amylopectin in nongranular form can interact to a greater extent with surfactants than a waxy starch, as supported by the findings of Eliasson and Ljunger [261], because surfactants have been shown to have a greater effect on decreasing the retrogradation of amylopectin gels than waxy starch gels. The amylopectin used by Gudmundsson [160] was from potato, which has longer outer branches than amylopectin from waxy maize starch [26].

The minimum chain length for the amylose molecule to form complexes has been reported to be 20 to 40 glucose residues when complexed with butanol [250]. Studies on synthetic amylopectin showed that a minimum of 15 to 20 glucose units of the outer branches was needed to complex with iodine at low temperatures [263]. The average chain length of the outer branches of amylopectin has been reported to be 16 to 20 glucose residues, depending on the botanical source [26]. In theory, there is no reason why outer branches with chain lengths longer than 20 glucose residues could not take part in complexes with a suitable compound with optimal chain length.

Other authors [160,259] have provided further evidence for amylopectin–surfactant complex formation, as both nongranular amylopectin [160] and waxy starches [171] with surfactant added (CTAB and/or SDS) showed V-type x-ray diffraction lines. When the amount of surfactant (SDS) was increased in the case of nongranular amylopectin, the V-type x-ray pattern lines increased in intensity, indicating that stronger or more interactions took place.

### 10.6.1.5 Macromolecules

A special situation arises when proteins or polysaccharides are mixed with starch as these components might themselves form gels. The compatibility between the components is very important and governs the rheological properties of the mixed system. Different types of gels might be formed, as described by Cairns et al. [264]. For two different polymers, the following types of gels were described: (1) a single polymer network containing the second polymer within the gel, (2) interpenetrating networks, (3) phase-separated networks, and (4) coupled networks. The influence on rheological properties will certainly differ depending on the type of structure formed. In a composite system, such as a starch gel, it is possible that another macromolecule influences the viscoelastic properties of the continuous phase. The
adhesion between the dispersed phase (the starch granules) and the continuous phase can also be affected when another macromolecule is introduced.

10.6.1.5.1 Proteins

The influence of proteins on the gelatinization of starch has been studied with the DSC, but in most systems the interpretation is difficult because the protein denaturation endotherm and the starch gelatinization endotherm overlap [265,266]; however, gluten is one protein for which it is possible to investigate the interaction [267]. Gluten by itself shows no, or at least very minor, transitions [268,269]. When gluten was added to wheat starch, an increase in \( T_o \) with increasing amounts of gluten was measured [267]. The thermal transitions in a mixture of starch and fish protein were found to proceed independently of each other [265], and in a surmi system the \( T_o \) for starch was shifted to a higher temperature compared with the starch–water system [270].

The influence of gluten on the rheological properties of starch gels has been investigated, and it was found that the effect of adding gluten depends on the type of starch to which it is added [119]. For mixtures of gluten and wheat starch, a weakening of the starch paste or gel in the presence of gluten was observed [270a]. The rheological behavior of starch–caseinate mixtures was studied in steady shear [271]. The mixed gel showed a shear-thinning behavior, as did the starch. A synergistic effect was found, as the starch–caseinate mixed gel showed higher apparent viscosities than did the single component. For mixtures of pea starch and egg white heated together, the modulus at small deformations increased for the mixtures compared with any of the components alone [272]. This was also the case for mixtures of amylose and egg white. Microscopic examination revealed a phase-separated structure in both systems. For mixed protein–starch gels (potato starch, annealed potato starch, pregelatinized potato starch, or cassava starch and bovine serum albumin [BSA] or gelatin) it was found that both the transition temperature and the rates of gelation of the components were critical for the behavior of the complex system [266]. When the starch gel was formed before the protein gel (e.g., BSA and cassava), \( G' \) and \( G'' \) of the complex system could be predicted by the simple addition of the moduli of the components at corresponding concentrations. When the gelation occurred in the reverse order (e.g., BSA and annealed potato starch), the gels were considerably stronger than predicted by simple addition. When starch is present in surmi, the rigidity of the mixture at increasing temperature is higher for the surmi without starch [270].

Another aspect of the gel formation of starch and starch components is the influence on diffusion of molecules in the gels. This effect was studied for the diffusion of BSA in amylose and amylopectin gels [273]. The diffusion coefficient of BSA in amylose and amylopectin gels was found to decrease with increasing polysaccharide concentration. No difference between amylose and amylopectin gels was observed.
Because starch is present as particles in most products, a starch interface exists and the starch can be regarded as a solid phase. The adsorption of proteins on starch has been studied, and it was found that BSA is adsorbed to a very low extent and wheat storage proteins are adsorbed to a much higher extent [78]. Differences between starches were also observed; for example, the adsorption of wheat storage proteins was much higher on potato starch than on wheat or maize starch.

It has been suggested that the interaction between starch and protein determines the endosperm hardness in cereals [39, 274]. The phenomenon has even been attributed to a single protein, friabilin [69], which has been suggested to influence the desorption of protein from starch during the starch preparation procedure [275]. Interactions between amylose or amylopectin and the protein oryzin were found to decrease during storage and were related to the stickiness of cooked rice [276]. The binding of oryzin to the starch components had a positive influence on the stickiness of cooked rice.

The presence of glass transition temperatures when heating 1:1 mixtures of amylopectin and gluten or amylopectin and casein was used to study the miscibility of these polymers [277]. It was evident from the presence of two separate $T_g$ values that amylopectin and gluten are immiscible, whereas the results were not as conclusive in the case of amylopectin and casein due to their similar $T_g$ values.

The molecular interaction between amylose or amylopectin and protein in solution has not been investigated to a great extent, except for some studies that have used the iodine-binding property of starch for detecting interactions [278, 279]. It was concluded that the association between wheat starch and wheat proteins occurs at neutral and acidic pH values. When starch, proteins, and lipids are all present, many interactions are possible. Three-component interactions were demonstrated in sorghum starch, whey proteins, and free fatty acids [279a]. This was observed as a cooling stage viscosity when all three components were present in the RVA. Certain proteins are lipid binding (e.g., whey proteins). The expected interactions between the starch and the lipids might then be cancelled because the lipids are bound by the protein and thus not available for complexation with amylose or amylopectin [279b, c].

### 10.6.1.5.2 Polysaccharides

Various cellulose derivatives (carboxymethylcellulose, microcrystalline cellulose, and alkaline soluble fibrous cellulose) were found to increase the retrogradation (DSC and rheological measurements) of sweet potato starch when added at a 9:1 starch/cellulose ratio [280]. Methylcellulose, on the other hand, was found to decrease retrogradation.

When gums (xanthan, carboxymethylcellulose, guar) were added to wheat starch the temperature of the initial viscosity increase was found to decrease from $83^\circ C$ for wheat starch to $53$ to $54^\circ C$ for mixtures with 0.45% gums added.
Peak height and viscosity after cooling also decreased. Apparent viscosities for mixtures of wheat starch (5%) and guar gum (0.5%), measured at different temperatures, were found to be higher than for starch alone [282]. The mechanical spectra showed that $G'$ increased when guar gum, locust bean gum, or xanthan gum was added to maize starch [283]; however, they were less elastic than the pure starch. For gellan gel, $G'$ increased with temperature when rice starch was added [193]. The greatest increase occurred at 65 to 75°C (i.e., when the starch gelatinized). The gelatinized starch thus caused a larger reinforcement of the gellan gel than did ungelatinized starch. This could be because the increase in starch granule volume depletes the gel phase of water or might be a result of improved adhesion between filler and matrix. Stress and strain at failure were decreased for the gellan gel when the rice starch was added [193]; however, stress as well as strain were found to increase with starch concentration after the initial decrease for gelatinized starch. Hydroxypropylcellulose (HPC) was not found to affect starch gelatinization (birefringence, swelling, amylase leaking, DSC endotherm) when added to wheat starch [284]; however, it is possible that the decreased precipitation of HPC in the presence of starch is due to adsorption of HPC on the starch granule surface. A mixed gel of wheat starch and ethyl (hydroxyethyl) cellulose showed more stability in rheological properties with time than a pure wheat starch gel [285]. It has been shown that amyllose might crosslink hydrophobically modified cellulose derivatives by forming inclusion complexes with the hydrophobic side chains of the cellulose derivative [285a].

10.6.2 Genotypes

Amylose constitutes 10 to 30% of the nonmutant normal starches; however, some cereals such as maize, sorghum, rice, and barley, have mutants that have starches with essentially 100% amylopectin — the so-called waxy starches. Some waxy starches do, however, contain a small amount of amylase (0.0 to 8.3%). The waxy barley starches contain the highest amount of amylase. The waxy cereal starches also contain lipids, but to a much less extent than their normal genotypes [286]. Barley and maize also have mutants that contain starches with unusually high amylase contents (40 to 70% amylase) [26,287]. New genotypes of wheat and potato have also been produced [29a,29b,30,31,99a,200a].

The gelatinization properties of a starch depend on its botanical source. The gelatinization enthalpies have been related to the degree of crystallinity in starch granules and the gelatinization temperatures to the degree of perfection of crystallites [42,98,288]; however, because crystallinity is primarily a property of the amylopectin molecule, a starch with high amylopectin content should have higher gelatinization enthalpy. In fact, waxy starches generally have higher gelatinization enthalpies than other starches, and amylomaize
Carbohydrates in Food

(45% amylopectin) has the lowest gelatinization enthalpy [170,172]. The gelatinization temperature should also be related to amylopectin content; however, the gelatinization temperature reflects not only the degree of perfection, or amount of crystallites, but also the glass temperature of the amorphous regions, which controls the onset of gelatinization. Amylomaize has a very high gelatinization temperature but a low degree of crystallinity. This is probably because of unusually stable glassy amorphous regions, as the amylopectin of the amylomaize has unusually long average chain lengths [49,289]. Waxy rice starchy with high gelatinization temperatures have greater swelling power and lower polysaccharide solubilization than waxy rice starchy with low gelatinization temperatures [27]. These waxy rice starchy contain amylopectins differing in molecular weight and shape; waxy rice starchy with high gelatinization temperatures have higher molecular weights [290].

The amylose/amylopectin ratio has a great influence on the rheological properties of pastes and gels. The gelatinized starch forms pastes and gels that can be considered as composite materials, where the dispersed phase is the swollen granules or remains of granules embedded in a continuous phase of amylose or polysaccharide matrix [146,153]. The amylose is the predominant gel-forming polymer, but the gelatinized granules contribute greatly to the mechanical properties of the gel [147,152,291]. Starches with more swollen granules exhibit a higher viscosity than starchy with less swollen granules at low concentrations [114,154,155]. The swelling of starch granules has been attributed to the amylopectin fraction [29]. Waxy starchy usually swell to a greater extent than normal starchy, and in normal cereal starchy amylose as well as lipids seem to retard the swelling of the granules [29]. The waxy starchy contain little or no amylose, so they form only viscous pastes or very weak gels because they build a very poor continuous polysaccharide matrix [171,172]. High levels of soluble amylopectin have also been shown to be detrimental to gel formation and reduce elasticity [187]. High amylose varieties, on the other hand, show very poor swelling when heated to 95°C at atmospheric pressure. For gel formation to occur in these starchy, autoclaving or jet-cooking is necessary. The retrogradation behavior of starchy from different genotypes was discussed in Section 10.4.

10.6.3 Chemical Modification

Starch could be modified in different ways by changing the chemical structure of the molecules. Three distinctively different methods can be identified:

- Hydrolysis, by acid or by enzyme
- Substitution, in which new groups are introduced into the molecules and monofunctional chemicals are used
- Crosslinking, in which two starch chains are crosslinked through a chemical bond and difunctional chemicals are used
The derivatization of starch is performed for several reasons (see Table 10.5), and in many cases the new starch is not going to be used in food products but for other industrial uses. All the goals listed in Table 10.5 cannot be obtained with one type of modification. Usually, only one or two properties are improved by a modification, and more than one modification might be required for an improvement to be achieved. Various modes of chemical modification are listed in Table 10.6.

To characterize the level of modification, the degree of substitution (DS) or molar substitution (MS) is used. DS is the average number of hydroxyl groups on each D-glucopyranosyl unit that are derivatized by substituent groups, and MS is the moles of substituent per D-glucopyranosyl unit. Because three hydroxyl groups in each D-glucopyranosyl unit are available for substitution, the DS value cannot be higher than 3. If the substituent group reacts further to form a polymeric substituent (i.e., more than one substituent on each available hydroxyl group), this can be expressed with the MS. This value gives the moles of monomeric units per mole of D-glucopyranosyl unit, and the MS value can be greater than 3.

One interesting question is, of course, where in the starch granule the chemical modification occurs. It is reasonable to think that the molecules in amorphous parts react first, but the reaction is not limited to these parts. Bromine oxidation of potato starch showed that the crystalline domains are also affected [293]. This also means that not only is amylose modified but also part of the amylopectin molecules. It has been suggested that in the case of amylopectin the modifications occur close to the branching points [205].
TABLE 10.6
Chemical Modification of Starch: Types of Modifications, Properties of Modified Starches, and Their Use in Food Industry

Oxidation
*Reaction achieved:* Depolymerization
*Properties:* Low-viscosity, high-solids dispersions; resistance to viscosity increases upon gelling in aqueous dispersion
*Use:* Lightly oxidized starches in batters and breading

Crosslinking
*Reaction achieved:* Introduction of intermolecular bridges by multifunctional reagents
*Properties:* Restricted swelling of the granule during gelatinization, resistance to shear, high temperature, and low pH
*Use:* Continuous cookers, sterilization, canning

Esterification
*Reaction achieved:* Introduction of acetate groups
*Properties:* Hydrophobic, cationic, or anionic character; prevents or minimizes association of outer branches of amylopectin molecules; prevents cloudiness and syneresis; promotes viscosity stability and clarity at low temperatures
*Use:* Canned, frozen, baked, and dry foods

Hydroxyalkyl starches
*Reaction achieved:* Introduction of hydroxyethyl or hydroxypropyl groups
*Properties:* Dispersion stability; nonionic character; decreased gelatinization temperature; low-temperature stability
*Use:* Low-temperature food storage conditions

Starch phosphate monoesters
*Reaction achieved:* Introduction of phosphate groups
*Properties:* Clarity; high viscosity; long, cohesive texture; stability against retrogradation
*Use:* As emulsifiers

Cationic starches
*Reaction achieved:* Introduction of a positive charge through tertiary amino or quaternary ammonium groups
*Properties:* Decreased gelatinization temperature; improved stability and clarity of dispersions
*Use:* Not used in food

Microscopic investigations have revealed that in the case of hydroxypropyl potato starch granules the modification occurs mainly in the central region of the granule [294].

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10.6.3.1 Gelatinization Behavior

The gelatinization temperature of modified starches is affected, either because that is the aim of the modification or because it is an inevitable consequence. DSC has been used to study the influence of chemical modification on the gelatinization parameters, and for most modifications a decrease in $T_o$ and $T_m$, as well as in $\Delta H$, has been found. These findings have been observed for hydroxypropyl distarch phosphates (maize, waxy maize, and tapioca) [295], for hydroxypropyl potato starch [296], and for a range of modifications of wheat starch (including hydroxyethyl and hydroxypropyl, acetate, distarch phosphate, aluminum octenyl succinate, and acetylated starches) [297]. In some cases (i.e., oxidation and crosslinking) an increase in $T_m$ was observed [201,297]. For an acetylated high-amylose starch, $\Delta H$ was found to increase compared with the native starch [201]. The changes in DSC parameters observed will increase with increasing MS, as has been observed for hydroxyalkyl starches [296–298].

When the DSC thermograms of a modified starch and its native counterpart are compared, the profiles look very much the same at high water contents [201,298]; however, when the measurements are performed at limited water levels (volume fraction of water = 0.55), some interesting results have been obtained [296]. At this intermediate water content, a biphasic gelatinization endotherm was observed for the native starch. The peak at the low temperature side of the double endotherm was found to decrease in size with increasing MS for hydroxypropylated potato starch. This was interpreted as being due to a change in the conformation of the starch chains in the amorphous regions and thus a change in the influence of the amorphous regions on the melting of the crystallites.

10.6.3.2 Rheological Behavior

One reason for the use of modified starch can be that a more viscous or more stable paste is required (see Table 10.5). Changes in rheological properties are thus notable, and several examples are available. Peak viscosity as well as setback values were increased for a hydroxypropyl distarch phosphate waxy maize compared with its unmodified counterpart, and acetylated distarch phosphate smooth pea and acetylated smooth pea both gave improved viscosity curves compared with the unmodified starch, whereas distarch phosphate smooth pea starch gave somewhat lower viscosities [205]. For hydroxypropyl potato starch, it was found that the pasting temperature and the peak temperature both decreased with increasing MS, whereas peak viscosity increased. The setback values were rather similar [294]. Crosslinking increased the viscosity of potato starch and waxy maize starch, but the concentration at which a measurable viscosity was obtained was also increased [155]. Fundamental rheological measurements have shown that a crosslinked waxy maize starch
gave $G'$ values similar to a normal maize starch, whereas the unmodified waxy maize starch gave very low values [201].

### 10.6.3.3 Cold Storage and Freeze–Thaw Stability

A critical test for the modified starches is the improvement of freeze–thaw stability. Also, the behavior during cold storage is of importance. It has been observed that storage of a starch gel for 7 days at 4°C results in more syneresis than one freeze–thaw cycle [205]. That the starch gel is not stable enough for cold storage or freezing can be observed by the liquid exudates (syneresis), by changes in the rheological properties of the gel, and by the presence of a DSC endotherm due to retrogradation of amylopectin [295,299]. The degree of syneresis has been observed to decrease with increasing degree of substitution for several modified starches (e.g., hydroxypropyl potato starch, acetylated smooth pea starch, acetylated distarch phosphate smooth pea starch gels) [205,207]. In relation to the syneresis, it is of interest to notice that the water-binding capacity (measured as unfreezable water using DSC) decreases with most modifications, the only exception being pregelatinized starch [300]. Very small, or no, endotherms were observed after storage for 7 days at 4°C for hydroxypropyl distarch phosphates [295]. Similar DSC results were obtained after 10 freeze–thaw cycles. Also, for hydroxypropyl potato starch, the DSC endotherm due to retrogradation was found to decrease with increasing MS [208].

### 10.6.3.4 Interactions with Other Components

Because the starch molecules (especially amylose) are modified due to the chemical modification, it is to be expected that molecular interactions such as the formation of the amylose–lipid complex change with modifications. When the interactions between monomyristin and hydroxypropyl potato starch were investigated, it was found that the DSC endotherm due to the amylose–lipid complex could be observed only at the lowest MS value (0.045). At higher MS values (0.125 or 0.170), no endotherm was observed [301]. For acetylated high-amylose maize starch, it was found that interaction with the surfactant (CTAB) gave lower transition temperature and enthalpy values than the modified high-amylose maize starch [201].

### 10.7 CONCLUSIONS

During the last decade or so, important achievements have been made in the starch research area. These include the arrival of new techniques such as the DSC $^{13}$C-CP/MAS NMR, and an increasing use of x-ray diffraction techniques and fundamental rheological methods. Another important development is the polymeric approach to interpreting starch behavior which encompasses the
glass transition concept and the description of the starch gel as a composite material. For the future, several challenges still must be met. The structure of the starch granule is still not known in detail. Interactions between starch and other components, especially macromolecules, have only just begun to be explored. Perhaps the biggest challenge is to relate our knowledge about the physicochemical properties of starch with our chemical knowledge about amylose and amylopectin in order to create tailor-made starches with the desired properties, both functional and nutritional.

REFERENCES


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11 Starch: Nutritional Aspects

Inger Björck

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11.1 INTRODUCTION

In most mixed diets, starch constitutes the main source of carbohydrates. Current dietary guidelines advocate an increased intake of available carbohydrates and dietary fiber and a concomitant decrease in fat. Put into practice, this would stimulate the consumption of starchy foods and further emphasize the quantitative dominance of starch in our diet; therefore, the potential qualitative differences in the nutritional properties of starch have become increasingly important. This chapter focuses on some nutritional parameters related to the availability of starch for digestion and absorption in the upper gastrointestinal tract and its fermentability in the large bowel.

11.2 NUTRITIONAL PARAMETERS

11.2.1 RATE OF SMALL INTESTINE STARCH UPTAKE

A quality concept based on the presumed effects of starch and other carbohydrates on blood glucose response was introduced at the beginning of the last century. In contrast to the low-molecular-weight carbohydrates, the starch polymer was considered to require a longer time for digestion in the small intestine, hence evoking low responses in blood glucose after a meal. This was considered of particular value in diabetics. As a consequence, starchy foods were classified as more beneficial in terms of blood glucose regulation in diabetic subjects (“slow” carbohydrates) than the low-molecular-weight sugars, which were believed to cause elevated glucose responses after a meal (“rapid” carbohydrates).

This classification of carbohydrates, based mainly on the molecular weight of the carbohydrate component, was never verified experimentally. Along with carbohydrate restriction, the belief that starchy foods were slowly digested and absorbed prevailed in dietary guidelines for diabetics during the 1980s, despite the pioneering work by Otto [1] and Crapo et al. [2,3] that challenged
the former classification of starchy foods. Results began to accumulate that suggested that starchy foods differed considerably with respect to their effects on blood glucose response [4–6], no matter their molecular weight. It was demonstrated that, despite their large molecular weight, soluble starch molecules were rapidly digested and absorbed in the small intestine [7,8], indicating that the rate of glucose absorption rather than digestion is the rate-limiting step for glucose delivery to the blood. However, despite an apparent surplus in the gastrointestinal tract of amylase, as a component in foods, several factors may obstruct the rate of digestion and absorption of starch, hence reducing metabolic responses. Today it is well established that starchy foods display features in the entire range from “slow” to “rapid.” Research during the 1990s verified a number of food factors that moderate the glycemic responses to starch in foods [9–11].

The effects of starch and other food carbohydrates on blood glucose response and insulin demand were originally considered only in diabetics. Today, the physiological impact of differences in postprandial glycemia to various foods is increasingly being discussed in relation to other metabolic disorders, as well as in healthy subjects. Although we still lack knowledge, there is evidence to suggest that most individuals probably benefit from a diet characterized by “slow-release” starchy foods [12] (see Table 11.1 and Section 11.4.2).

### 11.2.2 Extent of Small Intestine Starch Uptake

The previously held opinion of starch as a completely available source of carbohydrates has also recently been reconsidered. It has long been known that certain starches are poorly digested by amylases in the raw state [13–15]; however, until the mid-1980s, starch in heat-treated, ready-to-eat food items was considered completely digestible in the human small intestine. The occurrence of starch malabsorption in adult men was considered extremely rare [16]. This opinion was formed mainly based on the lack of reports concerning

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**Table 11.1**

**Potential Beneficial Effects of Slow-Release Starchy Foods**

- Facilitated blood glucose control in diabetics
- Reduced blood lipids in hyperlipidemic and healthy subjects
- Prolonged physical endurance during exercise
- Prolonged satiety
- Reduced cariogenic potential
- Protection against diseases linked to the metabolic syndrome (?)
- Delay of aging (?)
recovery of starch in feces; however, such findings cannot be taken as verification of the complete digestion and absorption of starch. Starch entering the large bowel may be fermented and utilized as a source of energy by the colonic microflora [17,18]. Moreover, certain starch fractions, which were later found to pass the small intestine in significant amounts, must be solubilized in either potassium hydroxide (KOH) or dimethylsulfoxide (DMSO) in order to be recovered in feces by common analytical procedures based on enzymic hydrolysis to glucose [19–21].

One of the first studies to show that starch in ordinary food items could pass through the human small intestine undigested was by Anderson et al. [17]. By quantifying the $\mathrm{H}_2$ in expired air after test meals as an indicator of colonic fermentation, they estimated that approximately 20% of the starch in white wheat bread entered the large bowel. At first, the delivery of starch to the large bowel was referred to as “starch malabsorption” [22]; however, by the use of similar indirect [23] or direct techniques (e.g., through quantification of the amount of starch passing the terminal ileum perfusion [24] or determination of starch in the ileostomy effluents from colectomized subjects [25]) it was shown that “malabsorption” of starch is a normal phenomenon.

The presence of an indigestible starch fraction was also detected \textit{in vitro}. Any dietary fiber method depends on amylase digestion to separate dietary fiber components from starch. In the early 1980s, it was shown that the dietary fiber content of potato or bread products was higher than estimated from the corresponding raw materials, due to the presence of a starch fraction that resisted analytical amylases unless solubilized in KOH [26,27]. At first, this was interpreted as an analytical nuisance that could be overcome by improving the efficiency of starch removal. Later, it was realized that the amylase resistance of starch in certain foods was indeed a nutritional entity, and its formation as a result of baking led to the expression “manmade dietary fiber” to describe this \textit{in vitro} indigestible starch.

During the last few years, much work has been performed to develop \textit{in vitro} procedures aimed at predicting the amount of starch entering the large bowel. Such starch (any starch or starch hydrolysis products passing the human small intestine) was defined as resistant starch (RS) [28]. In addition to differences in the rate of small intestinal starch digestion and absorption, the extent of starch uptake is also affected by various food factors, and a number of different RS fractions have been identified (see Section 11.5.2).

The amount of resistant starch in heat-treated foods varies considerably, from virtually zero to close to 40% on a starch basis [19–21,29–31]. The interest in RS from a nutritional point of view is not primarily related to a decrease in energy content, which at least with some products may be substantial, but rather to the potential effects mediated directly or indirectly in the large bowel (see Table 11.2 and Section 11.5.4). Except for the specific metabolic effects assigned to the viscosity of dietary fiber, RS can be expected...
to share physiological effects with those of other indigestible carbohydrates (dietary fiber, oligosaccharides from the raffinose family) or other carbohydrates that under certain conditions might escape small intestinal digestion or absorption in some individuals (e.g., lactose, fructose).

### 11.3 DIGESTION AND ABSORPTION OF STARCH

Starch digestion and absorption essentially consist of three phases: the intraluminal phase, the brush-border phase, and the glucose-absorption phase. The digestion of starch is initiated in the oral cavity by salivary α-amylase secreted from the parotid glands. Chewing also disintegrates the food, thus increasing the ratio of surface area to volume in the solid phase and hence enzyme accessibility. Despite the acidic conditions prevailing in the gastric juice, salivary amylase appears to retain some activity when passing through the stomach to the duodenum. Thus, the relative contribution of salivary amylase to the total amylase activity has been found to be approximately 15% in duodenal aspirates from healthy subjects [32]. Another α-amylase is secreted from the pancreas into the small intestinal lumen. Although slightly different with respect to structure and stability, the substrate specificity of these α-amylases is similar. The end products with amylose as the substrate are exclusively maltose and maltotriose. Degradation of amylopectin yields preferably maltose, maltotriose, and α-limit dextrins containing the α-1,6 branch links [33].

The degradation products from starch, with maltose dominating, diffuse from the lumen to the brush border of the small intestinal mucosa, where the final digestion to glucose takes place through the action of disaccharidases and oligosachharidases (sucrase–isomaltase complex, glucoamylase) with isomaltase, maltase, glucoamylase, and α-limit dextrinase activity. These “immobilized” enzymes are located close to the active transport sites, and the glucose formed is absorbed across the enterocytes by an Na⁺-dependent process into the portal blood. The glucose is then transported to the liver, enters
the circulation, and reaches peripheral tissues through the action of insulin. The hormone is excreted from the pancreas in response to an increase in blood glucose. The rise in plasma insulin will act to regulate the glucose level within a comparatively narrow range (glucose homeostasis) and stimulate synthesis and storage of glycogen in muscles and liver.

A reduction in the availability of starch to digestive amylases will reduce postprandial blood glucose and insulin response. The metabolic responses may also be lowered by, for example, a reduced rate of gastric emptying, a reduced motility of the luminal content, and a reduced rate of diffusion of starch hydrolysis products to the small intestinal mucosa. Such effects have been suggested to explain the blunting effect on postprandial glycemia of added viscous dietary fiber components [34] (see Section 11.4.3.1).

In infants with low or no pancreatic α-amylase secretion, starch has been shown to evoke a flat blood glucose response [35]. An optimal efficiency of starch digestion is therefore probably dependent on the delivery of easily diffusible α-amylolysis products to the small intestinal mucosa; however, when the total α-amylase activity was reduced to low levels in rats (or less than 1/10 of normal), the delivery of starch to the hind-gut increased from 6 to 9% of ingested starch [36], indicating a comparatively modest reduction in digestive capacity. It was found that patients who, due to pancreatic insufficiency, had a reduction in intraluminal hydrolytic capacity to 1/10 the normal level still retained an in vivo starch hydrolysis rate half that of normal [37]. This suggests that in the digestive channel of adult mammals α-amylase is present in surplus.

The efficiency of the digestive system in adults is substantiated by the fact that pure soluble starch produces a blood glucose response similar to that following ingestion of starch dextrins or glucose [8]. Viscous starch gels cause a rise in glucose response similar to that of glucose [38], and, in contrast to fiber polymers, the viscosity of starch decreases when passing the gastrointestinal tract. As judged from in vitro experiments with salivary α-amylase [39] and quantification of starch hydrolysis products during chewing [40], the viscous properties of starchy foods of a gel character are probably already significantly reduced during orogastric transit. Accordingly, no differences in the rate of gastric emptying were noted in healthy subjects given different gelled starches or a solution of glucose [41]. Less information is available concerning the importance of starch viscosity in intact foods. According to Gee and Johnson [42], the rate of starch hydrolysis, rather than the viscosity of partially digested foods, appears to be the main factor governing glycemic response. Thus, starch viscosity per se is unlikely to affect glucose response to any important degree, except perhaps in the case of subnormal α-amylase activities or in orogastric feeding where products may retain “starch-related” viscosity in the stomach [43]; however, the glycemic response to starchy foods may still be partially related to the rate of gastric emptying, and, in particular, an intact food form can be expected to prolong the retention time in the stomach [44].
Although some hydrolysis of starch by digestive enzymes probably takes place also in the colon, through the action of remaining luminal amylase activity, the digestive phase is completed. The further enzymic degradation and metabolism of resistant starch by microorganisms present in the colon are commonly referred to as fermentation.

### 11.4 POSTPRANDIAL BLOOD GLUCOSE AND INSULIN RESPONSES TO STARCHY FOODS

Several studies are available reporting blood glucose and insulin responses following ingestion of an equivalent amount of starch from various foods. In general, starch in bread, potatoes, and breakfast cereals produces high responses, whereas starch in legumes or products based on intact cereal kernels evokes small increments in blood glucose [2–6,45,46]. Starch in pasta and certain rice forms an intermediate group [4,47–51]. The differences in postprandial blood glucose response to some important sources of starch are evident from results in diabetic subjects by Collier and coworkers [52] (Figure 11.1). This variability in acute responses to starch emphasizes the need to classify product characteristics (see below).

Despite the prominent differences in postprandial glycemia when present in a food matrix, starch generally produces lower responses in blood glucose...
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than an equivalent carbohydrate load of glucose; however, there are exceptions. Certain “rapid” starchy foods, such as potatoes, corn flakes, and puffed rice, cause a glycemic response not very different from that of pure glucose [53]. It could be argued that this is because sucrose occasionally is added to commercial breakfast cereals. Although sucrose is a low-molecular-weight carbohydrate, the exchange of the “rapid” starch for sucrose in a meal with puffed rice actually lowered the metabolic responses in healthy subjects [54].

11.4.1 THE GLYCEMIC INDEX CONCEPT

11.4.1.1 Definitions

To allow for the ranking of products with respect to their influence on postprandial glycemia, the glycemic index (GI) was introduced by Jenkins and coworkers [4]. The GI is defined as the postprandial incremental area under the blood glucose response curve of a 50-g carbohydrate portion of a test food expressed as a percent of the response to the same amount of carbohydrate from a standard food taken by the same subject. Originally, 50 g glucose was used as the reference product, but later Jenkins and coworkers introduced white bread as the reference product [6]. With starchy foods, food portion size is usually standardized to provide 50 g starch. Depending on the analytical procedure used to quantify starch, RS may or may not be included as an available source of carbohydrates (see Section 11.5.4.3).

A source of some confusion is that either healthy or diabetic subjects may be included in the test. According to Wolever et al. [55], this should not obscure ranking, and, except for a higher variability in results from subjects with insulin-dependent diabetes mellitus (IDDM), subject characteristics do not appear to have a significant influence on the mean GI values of foods. Usually, 3-hour postprandial blood glucose areas are used when calculating GI values in diabetics and 2-hour areas in healthy subjects [56].

The time period used is probably not critical in diabetics (see Figure 11.1) but may significantly influence calculated GI values for certain products in healthy subjects [51]. A shorter time period (1.5 hour) was therefore recommended in healthy subjects to allow differentiation between starchy products causing a rapid post-peak decline (white bread) and products from which the starch is digested and absorbed over a longer period (pasta), thus leading to a low but sustained net increment in blood glucose in the late postprandial phase [51] (Figure 11.2).

Objections to the GI concept have been raised regarding methodology and interpretation as well as its application to mixed meals. These criticisms, however, have been adequately refuted [55], and several studies have now shown that the GIs of mixed starchy meals can be predicted from the GIs of the constituent foods [52,54,56,57].
11.4.1.2 Glycemic Indices of Starchy Foods

The glycemic indices of a number of starchy foods are provided in the literature. With glucose as the reference, reported GIs range from about 10 for starch in certain legumes to close to 100 in certain potato or rice products and breakfast cereals [10,58,59]. Published values with white bread as the reference product, or adjusted so the GI of white bread equals 100, cover a wide range, from 12 in the case of starch in bengal gram dal to close to 130 in baked potatoes or corn flakes [53].

The GI intervals for different groups of starchy foods, with white bread as the reference product, are displayed in Figure 11.3. Whereas most flour-based bread products produce high responses of glucose and insulin, pumpernickel-type bread products (based on intact kernels or baked at a lower temperature for a longer time) [4,60–64] or sourdough fermented bread [64,65] produced lower glucose responses than white wheat bread. The GIs of wholemeal bread products (wheat, rye, or barley) fall within a comparatively narrow range, close to that of white bread (88–103) [53,60–64], but the GIs of kernel-based bread range from 33 to 93, depending mainly on the proportion of starch enclosed within the intact kernels [60,61,63]. Apart from whole meal bread, many breakfast cereals (e.g., corn flakes, puffed rice, shredded wheat, muesli, and rolled oats) give high GI values ranging from 89 to 132 [53,66–68]. Reported GI figures for porridge oats differ; whereas some report a GI of 60...
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[67], others indicate a high GI of about 90 [68], not significantly different from white wheat bread. GI values for potato products are found in the upper range, from 80 to 128 (53,59); however, boiled potatoes appear to produce lower responses than baked or instant potatoes. In fact, several factory-processed foods (e.g., corn flakes, corn chips, puffed rice, instant potato) result in higher glucose and insulin responses than their conventionally cooked counterparts [59].

Important differences are reported for rice, with GI values varying from about 54 to 120 [4,58,59,69,70] depending on, for example, the type of rice (parboiled or regular), the amylose/amylopectin ratio, and the extent of cooking. According to Wolever et al. [69], the major cause of differences is the method of heat treatment: Parboiled or instant rice gave GI figures in the lower range around 65, which is in agreement with other reports [70]. In other studies [58], parboiled rice also gave high GI values, similar to glucose, and only high-amylose rice produced a significantly lower GI among the 12 varieties studied.

Although the features of boiled pasta products appear to be more homogeneous [47–51], differences do exist [48]. The range in GI of boiled pasta products is from 38 in the case of protein-enriched spaghetti to 70 in ordinary spaghetti and macaroni products [48,50,51,53]; however, canned pasta increased the incremental blood glucose area by a factor of two compared with boiling [71], suggesting a detrimental impact of cooking at elevated temperatures.
Most legume products prepared by conventional cooking produce low glucose and insulin responses [45,66,72]. Differences in GI characteristics are, however, also evident from results with legumes, for which the GIs range from 12 to 74 [53,66]. Apart from botanical differences, processing involving canning [73–75] or mechanical disruption [75–77] appears to increase GI values.

One obstacle when interpreting GI data is that the products studied are sometimes poorly characterized as to their composition and conditions of processing. In light of the importance of both these characteristics for determining the properties observed, the need exists for an extended list of GI data from well-characterized products. Such a list is necessary to allow full evaluation of the potential advantageous effects currently discussed in relation to low-GI foods (Table 11.1)

### 11.4.2 Metabolic and Health Implications of Low- vs. High-GI Starchy Foods

#### 11.4.2.1 Importance in Relation to Glucose and Lipid Metabolism

Data from a number of medium- to long-term studies in diabetic subjects suggest that low-GI diets improve parameters related to glucose and lipid metabolism. Due to the quantitative importance of starch in the diet, the GI characteristics of the diets tested reflect mainly those of the starchy components. Reported effects of low-GI diets include reduced glycosylated hemoglobin, fructosamine, and day-long blood glucose levels, all of which are indicators of improved glycemic control [54]. Low-GI diets also reduced serum low-density lipoprotein (LDL) cholesterol and triglycerides in diabetics [78,79]. A reduction in blood lipids was seen also in hyperlipidemic patients with diets based on low-GI starchy foods [80], the effect being most pronounced in subjects with hypertriglyceridemia.

Possible mechanisms by which low-GI diets affect these parameters have been discussed in relation to studies in which meal frequency was altered (nibbling vs. gorging) to mimic differences in the rates of starch digestion and absorption [81]. A crucial parameter is probably the lowered insulin secretion that may implement changes leading to, for example, a reduced activity of cholesterol-synthesizing enzymes or an improved insulin sensitivity [81]. Products with a high RS content may also enhance glucose tolerance through the short-chain fatty acids produced from colonic fermentation [82] (see Section 11.5.3). Although the potential of low-GI foods appears particularly great in the management of hyperlipidemia, modest reductions in dietary GIs have also been shown to improve metabolic control in diabetes. It has been said that the “time has come to reassess the value of GI in planning meals for diabetics” [54].

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The GI characteristics of starch in the diet may also have implications for healthy subjects. It has thus been suggested that low-GI starchy diets may protect susceptible populations from developing non-insulin-dependent diabetes [72,83]. A low-GI diet was also shown to reduce 12-hour blood glucose profiles and lower insulin secretion in healthy subjects as well as total plasma cholesterol [84], suggesting a protective role against the development of diseases linked to the metabolic syndrome. In light of the potential role of glycosylation of hemoglobin and other body proteins in the aging process, such a diet might delay this process [12].

11.4.2.2 Physical Performance

A few studies are available that discuss the importance of the GI characteristics of starchy foods in relation to physical endurance during strenuous exercise. In a study by Thomas et al. [85], a cyclist pedaled to exhaustion following test meals based on either a high-GI (potatoes) or a low-GI (lentils) food. The endurance time was significantly longer (20 min) following the low-GI meal, possibly due to the maintenance of a sustained plasma glucose level during exercise. Beneficial effects of the pre-event consumption of low-GI breakfast cereals, in terms of a higher blood glucose level after 100 minutes of exercise, were also reported [86]. In contrast, as judged from the analysis of muscle glycogen in cyclists following prolonged exercise, the repletion of glycogen stores was more efficient with high-GI foods [87].

11.4.2.3 Satiety

A number of factors influence hunger and satiety after a meal. Suggested physiological mechanisms of importance with regard to the GI of starchy foods are the rate of glucose absorption and the rate of gastric emptying [88]; also, dietary fiber was early suggested as promoting satiety [89].

In a study by Holm et al. [62], bread with low GI characteristics was generally more satiating than white wheat bread; however, the more satiating products contained more dietary fiber, which may have affected the results, as bread with a higher dietary fiber content has been shown to produce a higher satiety score irrespective of the GI [63]. In more elaborate work on satiating effects by Holt et al. [67], a significant inverse relation was obtained between the GI and insulinenic index (II) and the peak satiety scores for six different starchy foods. The GI and II values were also inversely related to postprandial cholecystokinin levels. This hormone is secreted in the upper small intestine in response to a meal and may, among other effects, reduce the rate of gastric emptying. A prolonged duration of satiety was also observed following ingestion of a bean purée with cell-enclosed starch (low GI), compared with a corresponding purée based on potatoes (high GI) [90]. The rebound fall in blood glucose in the late postprandial phase with the rapid
potato meal was related to a more rapid return in hunger sensation as well as a more pronounced desire to “eat something tasty.” The low-GI product caused a significantly higher rating of stomach fullness, which might suggest that this product induced a slower rate of gastric emptying [90]. The impact of the rate of gastric emptying was also suggested in a paper by Liljeberg et al. [65], who found that the addition of sodium propionate to bread not only reduced but also delayed the postprandial peak glucose response in healthy subjects. This sodium-propionate-supplemented product also evoked a higher satiety rating.

The mechanism by which certain low-GI starchy foods promote higher postprandial satiety is not known. Apart from differences in the features of the starch in the test products, the test meals in many cases also differed in, for example, type and amount of other food constituents such as protein, dietary fiber, and fat. In a study of breakfasts based on spaghetti or white bread, both made from identical ingredients, no differences were noted in satiety nor in voluntary food intake at subsequent lunches, despite prominent differences in GIs; however, the insulin response to lunch was significantly higher following consumption of the “rapid” starchy breakfast [91].

More studies are obviously needed in this area. The food factors causing low GI features may possibly affect the probability of an impact on satiety; for example, for several boiled barley porridges the GIs were found to be inversely correlated with satiety ratings, but a flour-based porridge made from high-amylose barley deviated by evoking an unexpectedly high postprandial satiety [92]. A lower postprandial blood glucose response and higher satiety were also noted when the amylose/amylopectin ratio of autoclaved starch added to a lunch meal was increased [93]. The potential effects of resistant starch should be evaluated, because the most satiating products in these studies can be expected to have contained higher amounts of such starch.

11.4.2.4 Caries

Although sucrose is most potent with respect to its influence on the development of caries, starchy products also promote a postprandial pH drop in dental plaque [94]. The magnitude of this drop appears to be largely dependent on the enzymic availability of starch [95]; hence, a higher availability of starch to salivary α-amylase will promote a higher yield of maltose and other low-molecular-weight dextrins in the oral cavity. These dextrins form a substrate for fermentation by the plaque microorganisms, and the organic acids thus generated will eventually lower the plaque pH. Other factors of importance include, for example, starch stickiness and adherence to the tooth surface [96], as well as the oral retention time [97].

The drop in dental plaque pH in response to variously processed starchy products has been reported in several studies, either following a mouth rinse with suspensions or in realistic eating situations. Raw starch has been shown
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...to elicit a less pronounced drop in plaque pH compared with gelatinized starch, whether tested in the form of pure starch or suspensions of raw or processed products [98,99]. When the cariogenic potential of different starchy foods was evaluated in a realistic eating situation [100], the ranking of products based on the area under the pH curve was close to the ranking based on the GI concept. These results suggest that, with respect to cariogenic properties, starch foods cannot be regarded as a homogeneous group. Also, according to Glor et al. [101], starchy foods must therefore be considered, particularly in the case of sucrose-containing products, such as corn flakes, which were reported to lower plaque pH to the same extent as pure sucrose [99].

11.4.3 Food Factors Affecting Blood Glucose and Insulin Responses to Starch

During the last decade, a number of food factors have been identified that reduce metabolic responses (Table 11.3). Some factors are related to the inherent properties of the raw material whereas others are acquired during the food process. A major mechanism for differences in GI from starchy foods involves differences in the rate of digestion of starch [5,102–105]. Most of the food factors discussed below reduce the rate of hydrolysis. Differences in the rate of gastric emptying [44,106], the rate of diffusion of starch hydrolysis...
products, and the rate of glucose absorption in the small intestine may also play roles in the rate of delivery of glucose to the blood.

Solids empty from the stomach more slowly than liquids and are retained in the stomach until reduced to a particle size of <2 mm [107]. By increasing the amount of dextrinized starch already in the stomach, a higher rate of gastric emptying may in fact be linked to a higher availability to salivary α-amylase in the oral cavity and in the stomach. This could be one reason why the GIs of starchy food correlate not only with the rate of enzymic digestion as measured in vitro [5,102–105] but also with the rate of gastric emptying [44].

11.4.3.1 Dietary Fiber Per Se vs. Dietary Fiber in Relation to Food Structure

One of the first food factors discussed as a potential moderator of the glucose and insulin responses to carbohydrates in food was dietary fiber [8]. A number of studies have shown that the addition of viscous dietary fiber such as guar gum or pectin reduces postprandial glycemia response to a glucose load [108]. Starchy foods, particularly cereal products, are natural vehicles for enrichment with dietary fiber. Enrichment of bread products with viscous fiber such as oat or rye bran or linseeds also appears to blunt metabolic response [62,109], the effect being most pronounced on insulin response in the case of linseed fiber [62]. Guar enrichment of pasta either reduces glycemic responses in diabetic subjects [110,111] or has no impact [112]. Enrichment of fettucini with oat bran did not affect metabolic responses in healthy subjects [71], whereas a similar addition of oat bran to white bread significantly reduced the GI [62]. The lack of effect in some studies with pasta suggests that fiber enrichment might deteriorate the product texture (see Section 11.4.3.2.3), which is a food factor of prime importance for the low-GI properties of pasta. The type of starchy food selected for fiber enrichment could thus be critical.

As to the effect of naturally occurring fiber in foods, a number of studies are available reporting GI values for cereal products based on either white or unrefined ingredients. White or brown rice [4,9], white or brown spaghetti [47,49], or white or brown wheat bread [60,113] produce similar blood glucose responses. These cereal foods contain mainly insoluble dietary fiber; however, no effects were noted when wheat was exchanged for whole-meal rye or barley flours in bread products [60,64]. Reports on oat-based products, such as bread [63], muesli [68], or porridge [53,68], suggest that the glycemic responses are close to that of white wheat bread, despite the higher viscous dietary fiber content of the oat-based products.

The content of β-glucans has been reported to decrease during sourdough fermentation [114], possibly due to hydrolysis of the fiber polymers. In two studies reporting glycemic responses to whole-meal barley porridge [92] and
whole-meal barley bread [63], a reduction in response was noted in the case of the porridge, whereas the barley flour bread produced a glucose response similar to that of white bread. This difference might be due to the fact that \( \beta \)-glucanase, intrinsic to the barley material or from the yeast, hydrolyzes the \( \beta \)-glucans during dough making and fermentation. The intrinsic activity might possibly be destroyed during the boiling of a porridge product, thus maintaining a higher viscosity in the product. Inconsistent data are also reported for oat porridge [4,67,68]. At least with guar gum, it has been demonstrated that the granulation process used when preparing pharmaceutical preparations may reduce viscosity to such an extent that the preparation is rendered clinically ineffective [115]. It remains to be shown whether differences in responses to certain cereal products are linked to the ability of the food process to maintain the viscous properties of the fiber components or to differences in the amount or character of the polymers per se. Also, differences in the amylose/amylopectin ratio of the starch moiety should be considered (see Section 11.4.3.2.2).

In a review by Nuttall [116], it was concluded that the naturally occurring dietary fiber in foods per se is of little or no value in controlling plasma glucose values in diabetics. This is also the major conclusion from the discussion above; however, despite the modest effects of dietary fiber per se, as a component in cell walls and the botanical tissue the fiber network may significantly reduce the availability for amylolytic attack, thus reducing the rate of small intestinal starch digestion. This encapsulation of starch in a fiber network is probably the main cause of the reported relation between GI and total dietary fiber content [66].

The importance of maintaining the botanical tissue as intact as possible has been demonstrated in several studies. Milling of rice significantly increased the postprandial glycemia response to starch [9]. Whereas starch in bread products based on milled flours generally evokes high metabolic responses, bread products with intact kernels remaining have been shown to produce low responses of glucose and insulin [60–64]. In the case of scones, reports have shown a lower postprandial response to bread made from coarsely rather than finely milled flour [117,118]. Whereas porridges made from oat flakes or barley flours showed GIs ranging from 60 to 90 [67,68,92], products made by boiling intact cereal kernels displayed GIs in a lower range, from 30 to 70 [60,68,92]. The impact of the kernel-to-flour ratio on the GI features of bread products is displayed in Figure 11.4.

The importance of botanical structure has also been demonstrated with legumes. From legumes, precooked flours can be prepared that maintain a considerable portion of the starch enclosed within intact cells. When tested in mixed meals, a purée made from a white bean flour with remaining undamaged cells produced significantly lower incremental areas of glucose and insulin in diabetic subjects than a corresponding product from white bean flour with
damaged cells [76]. Similar results were obtained with products based on precooked red kidney beans with or without undamaged cells [75]; however, boiled intact red kidney beans displayed the most beneficial features. Apparently not only the botanical structure at a cellular level but also the tissue structure provide an enzymic barrier, and the glycemic responses have been found to correlate with the rate of enzymic digestion as measured \textit{in vitro} [75,119].

Apart from decreasing enzyme availability, an intact botanical structure, by increasing the “solid” phase in the stomach, might also reduce the rate of gastric emptying. The maintenance of intact cells may further restrict swelling of the starch [119], thus not only reducing the physical accessibility but also the enzymic availability of the substrate \textit{per se} (see Section 11.4.3.2.1).

It can thus be concluded that dietary fiber in relation to food structure is a more important entity than the content of naturally occurring dietary fiber \textit{per se}. Regarding the exceptionally low glycemic responses to legumes or foods based on intact cereal kernels, the botanical structure is probably of major importance for the beneficial features observed. In the case of legumes, however, the presence of other factors intrinsic to the raw material (see Section 11.4.3.3) or the generally higher amylose/amylopectin ratio (see Section 11.4.3.2.2) may contribute.

\begin{figure}
\centering
\includegraphics{figure114.png}
\caption{The importance of the ratio of intact kernels to flour for the glycemic index (GI) of barley bread (\(\triangle\), results from Liljeberg and Björck [64]; \(\bigcirc\), results from Jenkins et al. [61]). The GI of boiled barley kernels was included for reference (\(\bullet\), results from Granfeldt et al. [92]). (Adapted from Liljeberg, H. and Björck, I., \textit{Eur. J. Clin. Nutr.}, 48, 151, 1994.)}
\end{figure}
11.4.3.2 Factors Related to the Chemical and Physical Structure of Starch

11.4.3.2.1 Degree of Cooking/Gelatinization

Raw starch is only slowly hydrolyzed by amylases in vitro. As a result of gelatinization, the in vitro amylolysis rate increases dramatically [120–124]. In the case of several starches, this effect on enzyme availability is also evident in vivo. It is thus known that raw maize, wheat, manihot, and smooth pea starches or raw potatoes result in considerably lower postprandial responses in blood glucose than the corresponding boiled ingredients [102,125,126]. The slow rate of glucose delivery to the blood in the case of raw maize starch has been utilized clinically in patients with glycogenosis, who are dependent on a continuous exogenous supply of glucose to the blood, because this cannot be achieved from hepatic release [127]. Also, a diet characterized by raw foods has been shown to improve blood glucose regulation in diabetics [128].

With some exceptions, the starch in most ready-to-eat food items is more or less completely gelatinized. In studies with suspensions of wheat starch or variously processed wheat products [43,129], a correlation was obtained between the degree of gelatinization and postprandial glucose and insulin responses in rats following orogastric intubation; the lower degree of gelatinization, the lower the glucose response. A lower degree of gelatinization was also accompanied by a slower rate of in vitro starch digestion.

Even at a comparatively low degree of gelatinization (14%), however, glucose responses increase dramatically [124], suggesting that the gelatinization must be maintained at low levels to affect metabolic response to an important extent. The availability of starch to salivary α-amylase in the oral cavity was also shown to be influenced by the degree of starch gelatinization [99]. As a consequence, the pH drop in dental plaque following a mouth rinse with suspensions of variously processed cereal products was more pronounced at higher degrees of gelatinization.

As to the nutritional importance of intermediate degrees of gelatinization in intact foods, the conclusions are less clear cut. No correlation was obtained between GIs in healthy subjects and the degree of gelatinization for seven wheat products [130]. Despite intermediate levels of gelatinization, flakes produced by rolling steamed rye or oats, respectively, evoked high responses of glucose and insulin, similar to those with white bread [68,131]. In contrast, starch in rolled raw wheat kernels induced a comparatively small increment in blood glucose [132], again emphasizing that low levels of gelatinization are necessary to affect metabolic properties in cereal products. In one study, however, although a significantly lower GI was obtained following the rolling of raw oat kernels (GI, ~70) compared with commercial preheating and rolling conditions (GI, ~100) [133], this “raw” oat flake produced a higher glycemic response than anticipated from data with other raw cereals [102,126]. These
results imply that raw oat granules might be more susceptible to amylases than previously studied raw starches.

In products with remaining botanical integrity, this could also influence the degree of gelatinization; however, as judged from results with barley porridges, products based on intact kernels displayed low-GI features despite a complete degree of gelatinization [92]. Evidence suggests that more severe heat treatment conditions will render the product more rapid in character. The extent of cooking regular rice (5 or 15 min) significantly affected the incremental blood glucose areas in diabetic subjects — a prolonged cooking time increased metabolic response [69]. In contrast, no effect of cooking time (5 to 25 min) was noted with parboiled rice. Although it is possible that the longer cooking time for regular rice increased the degree of gelatinization, an increase in metabolic response could also have resulted from a disruption of the botanical structure [9]. Autoclaving of red kidney beans [75] or pasta [71] likewise increased metabolic responses in healthy subjects compared with boiling at atmospheric pressure.

More studies are needed to elucidate the potential of intermediate degrees of gelatinization and limited swelling of starch and whether these parameters can be used to moderate glycemic response to starch in realistic food items. Overall, cooking for extended periods or at elevated temperature causes a deterioration in the nutritional properties of the food by disrupting the food structure and increasing the extent of hydration of starch molecules.

11.4.3.2.2 Amylose/Amylopectin Ratio

The amylose/amylpectin ratio is higher in legume starches than in “common” varieties of cereal or tuber starch [134]. The low GIs reported for most legume products have focused interest on potential varietal differences in amylose content. Among cereals (e.g., rice, corn, and barley) the amylose/amylpectin ratio may differ considerably among genotypes. In some studies on rice [58,135,136], a higher amylose content was shown to lower metabolic responses. In fact, according to Brand-Miller, only high-amylose varieties of rice are potentially useful in low-GI diets [58]. By exchanging ordinary corn flour for high-amylose corn flour (70%) in arepas, glucose and insulin responses were significantly reduced in healthy subjects [137]. Results with rice genotypes, however, are not consistent, and in some reports no effect was noted due to amylose content [138]. In vitro measurements of the digestion rate in boiled rice from genotypes varying in amylose content are even more confusing. As a consequence, a higher amylose content in boiled rice was found to actually increase the rate of amylosis [139]. In contrast, according to our own observations in an in vitro system based on enzyme incubation of chewed samples, the rate of release of starch hydrolysis products from dialysis tubing was inversely related to the amylose content of the rice [140]. Sticky rice, with a low amylose content, was further hydrolyzed very rapidly in vitro,
producing a hydrolysis graph similar to that for white bread [104]. Also, the metabolic responses and in vitro rates of starch hydrolysis of gels made from wheat, manihot, and smooth pea starch were inversely related to the amylose content (17 to 35% amylose) [102].

The inclusion of high-amylose corn starch (70% amylose) in products has been shown to lower metabolic responses compared with products based on low-amylose starch. Behall et al. [141] reported reduced postprandial responses of glucose and insulin in healthy subjects following ingestion of crackers made from high-amylose corn starch compared with a corresponding product made from low-amylose starch. A beneficial effect of incorporating autoclaved high-amylose corn starch into products was further reported by van Amelsvoort and Weststrate [93] in healthy subjects. In both investigations, the effect on insulin was most pronounced, which is in agreement with data on high-amylose rice [135]. Inclusion of high-amylose corn starch also improved metabolic parameters such as mean fasting triglyceride and cholesterol levels during a 5-week study in healthy subjects [142].

The reason why a higher amylose content may improve metabolic response to starch in foods is not fully known. One suggested mechanism includes a lowered rate of amylolysis caused by incomplete gelatinization of high-amylose starches at ordinary cooking conditions [29,93,143]. As a consequence, it has been suggested that, provided rice is cooked at its minimum cooking time, thus resulting in the same degree of gelatinization, similar glycemic properties could be expected irrespective of amylose content [143]. A higher amylose content will increase the temperatures necessary to solubilize the starch and develop viscosity; however, as judged from differential scanning calorimetry (DSC), even high-amylose corn starch granules (70% amylose) are completely gelatinized below 100°C, also at intermediate moisture contents (water-to-starch ratios: 57 to 66) [144,145].

In the case of boiled suspensions of barley flours varying in amylose content from 8 to 35%, no differences were observed in the rate of in vitro amylolysis [146]. In contrast, following autoclaving of the same barley flours, the rate of enzymic digestion was significantly lowered in the high-amylose barley flour. This implies that the effect of a higher amylose content on enzyme availability was actually more pronounced at conditions that could be expected to facilitate swelling of starch granules. Another plausible mechanism for the lowering of enzyme availability is the retrogradation of amylose, which can be expected to be favored by autoclaving, particularly if performed in repeated cycles [147]. Even when an amylose-enriched high-amylose corn starch is used which could be “gelatinized” completely at normal cooking temperatures, inclusion of such starch into products has been shown to reduce insulin response to a breakfast meal compared with low-amylose starch [148]. Although the effect on the metabolic response was less pronounced than in the previous study with the addition of autoclaved “ordinary” high-amylose
corn starch [93], the effect on insulin was significant. This was despite the fact that the amyllose-enriched corn starch contained a lower amyllose content. The importance of incomplete gelatinization and the swelling of high-amylose starches to lowering the metabolic response should be further evaluated.

Apart from retrogradation, amyllose may also interact with other food components (e.g., lipids) [149]. Both phenomena can be expected to lower the rate of starch digestion (see next section). The potential effects of a lowered content of “available starch” in the products should also be considered, as a high amyllose content is known to favor a high content of RS; however, even when products were compared on a similar potentially available starch basis, high-amylose corn arepas still produced a lower glycemic response than a corresponding “ordinary” arepa product [137]. The high-amylose arepa contained an appreciable portion of resistant starch. Although incomplete swelling of starch cannot be excluded as a mechanism for RS formation and a reduced availability of the potentially available starch fraction, arepa making involves repeated heat treatment, which is known to favor amyllose retrogradation [147,150].

Whatever the mechanism, there are several indications of favorably low glucose and insulin responses to foods based on genotypes with a high amyllose content. This should be acknowledged when selecting genotypes for human consumption, and attempts should be made to produce palatable products from such raw materials.

11.4.3.2.3 Starch Interactions/Physical Structure

The importance of the interactions between starch and protein on the α-amylolysis rate has been discussed. In vitro studies have demonstrated that the protein matrix, in cereal [43,151,152] as well as in legume products [153], limits the accessibility of starch to amylase. By deproteinizing pasta, the rate of in vitro amylolysis increases [154]. Differences in the in vitro rate of amylolysis among processed wheat samples evened out following preincubation with pepsin [43,152]. The in vitro procedure, which included pepsin, allowed a closer prediction of glycemic response in rats, suggesting that at least some protein–starch interactions are broken at physiological conditions [43].

In pasta products, gluten forms a viscoelastic network that surrounds the starch granules, thus restricting starch swelling and leaching of starch during boiling [154,155]. As to the cause of the slow-release starch features of pasta, the limited swelling of starch granules may reduce the availability to amylases; however, as judged from a prominent increase in the rate of amylolysis even at pregelatinized stages of crystallinity [124], differences in the extent of swelling of gelatinized granules might not account for the lowered availability observed. The presence of a glutinous phase, although available to proteolytic enzymes, will possibly release the starch substrate more gradually to amylolytic attack. According to Jenkins [151], protein–starch interactions also
reduce the availability of the starch in bread products, and a bread made from gluten-free flour has been shown to elicit a higher glucose response than an ordinary wheat bread.

Other forms of starch interactions involve the formation of amylose–lipid complexes and interactions among starch molecules. As a consequence, amylose complexed with lysolecithin is more slowly digested and absorbed from the rat small intestine and produces less pronounced postprandial glycemia than does “soluble” amylose [156]. This reduction is noteworthy, as the soluble amylose reference can also be expected to be less readily available for amylases due to its disposition to retrograde. In products based on starches in which amylose is the minor starch component, amylose retrogradation and the formation of amylose–lipid complexes are probably most efficient in reducing the enzymic availability if the amylose engaged is enriched on surfaces, thus encapsulating the bulk of the starch, rather than being evenly distributed. Such interactions may explain the improvement in product characteristics of rice seen in some studies as a result of parboiling. The low glycemic responses reported for products based on high-amylose starches might, however, be related to retrogradation of amylose. Although high-amylose starch granules do not swell at “ordinary conditions for food preparation,” following the disappearance of the crystalline granule structure amylose molecules could still interact, leading to a reduction in the overall enzymic availability of starch. As to the effect of the retrogradation of amylopectin, even significant staling of starch in bread did not reduce the rate of in vitro amylolysis [157].

11.4.3.3 Antinutrients

Compared with cereals and tubers, legumes are rich sources of antinutrients such as phytic acid, polyphenols, and lectins. These substances are known to reduce the availability of protein and minerals in particular; however, their effects in relation to starch bioavailability have also been addressed. A number of studies have demonstrated the ability of phytic acid, tannic acid, and hemagglutinins to inhibit α-amylase activity in vitro [158–165] or bind to the starch substrate [166], indicating that these substances have the potential of interfering with starch digestion in vivo. In some of these studies, the amylase was exposed to the inhibitors at unphysiological conditions, particularly in vitro studies with sodium phytate, where alkaline conditions are obtained. As a consequence, the inhibitory effect essentially disappeared at neutral pH [161,164,167]. Other suggested effects of antinutrients include the inhibition of small-intestinal glucose absorption; thus, polyphenolic constituents in extracts from bean varieties were shown to inhibit glucose transport across the rat ileal mucosa in vivo [168].

With regard to the effects of starch on the rate of small intestinal digestion and absorption in humans, the addition of sodium phytate to unleavened bread significantly reduced glycemic area in healthy subjects compared with no
addition [158]. The glycemic response to unleavened bread made from navy bean flour was significantly lowered when prepared from undephytinized flour instead of dephytinized flour [169]. The addition of calcium to the undephytinized products led to an increase in glycemic response, suggesting that the inhibition of \( \alpha \)-amylase by phytate is mediated through the binding of calcium [158,169]. Apart from phytic acid, the presence of polyphenols and lectins has also been discussed with regard to their having a blunting effect on the metabolic response to starch. Thus, an inverse relation between polyphenol and lectin content in starchy foods and the magnitude of glycemic responses was reported in studies by Rea et al. [160] and Thompson et al. [170].

The relative importance of antinutrients in explaining the features of starch in legumes remains to be established. According to Wursch [119], the entrapment of starch within parenchyma cells, which physically obstructs enzymic access and limits starch swelling, was suggested as being a major cause of the beneficial properties. Nonetheless, although a mechanical disruption of the parenchyma cells in red kidney bean product significantly increased the blood glucose response in healthy subjects relative to a corresponding product with cell-enclosed starch, the bean product with released starch still had a GI of 76, which is significantly lower than that of white wheat bread [75]. This may indicate an impact of antinutrients, as well as the comparatively high content of amylose and the presence of viscous dietary fiber.

The strongest evidence for a blunting of postprandial glycemia is provided in studies with realistic products in which phytate was either removed [169] or added [158]. In these studies, the presence of phytate was inversely correlated with the glycemic response. Whether this effect can be assigned solely to amylase inhibition or to effects on the rate of gastric emptying, similar to those reported by other organic acids, remains to be established.

### 11.4.3.4 Organic Acids/Corresponding Salts

The possible influence of organic acids, either formed during the fermentation of foods, for example, or added, on postprandial glycemia in response to starchy foods is a comparatively recent topic. Interest originally was in the nutritional effects of the short-chain fatty acids (acetate, propionate, butyrate) produced during fermentation of indigestible carbohydrates in the colon. It was consequently suggested that these acids, when absorbed from the colon, have beneficial effects on glucose and lipid metabolism [29].

In a study by Todesco et al. [171], the incorporation of sodium propionate into bread significantly lowered blood glucose responses in healthy subjects (by approximately 40%). The rate of starch hydrolysis as measured \textit{in vitro} was significantly reduced in the presence of sodium propionate, suggesting an effect on the rate of \textit{in vivo} starch digestion. A beneficial effect on carbohydrate tolerance was also noted with a sourdough-fermented pumpernickel product containing appreciable amounts of acetic acid [64]; however, the starch in this
product was equally rapidly hydrolyzed by amylases in vitro as starch in white wheat bread, suggesting an effect other than amylase inhibition.

In a study on bread products containing equimolar amounts of added or spontaneously generated lactic acid (sourdough) vs. added calcium lactate, lactic acid but not calcium lactate reduced glucose and insulin responses in healthy subjects [65]. In vitro studies indicated a lowered rate of amylolysis in the case of products containing lactic acid, but not calcium lactate. A lowering of postprandial responses was, however, noted following the addition of sodium propionate to bread products, despite a lack of inhibitory capacity during in vitro amylolysis. Whereas most flour-based breads, including rye and barley bread, produce high responses of glucose and insulin, a lowered metabolic response was also noted in diabetic subjects following the ingestion of a sourdough-fermented rye bread [131].

Indications of the beneficial effects of organic acids are also evident from other reports; thus, the inclusion of fermented vegetables [172], vinegar-dressed salad [173], or vegetables with added lactic acid [174] in starch-based meals has been found to reduce the postprandial responses of glucose and insulin.

From the studies referred to above it can be concluded that the metabolic responses to starchy foods or mixed meals may be lowered by certain organic acids or corresponding salts. Possible mechanisms involve a reduced rate of gastric emptying or a lowered rate of starch digestion. A flattening of postprandial glycemia by various organic acids was also noted in rats in the case of glucose administration, for which potential effects on amylase activity can be excluded [175]. In that study and in others [176,177], organic acids were shown to delay gastric emptying. A suggested mechanism is the presence of receptors in the duodenum, which, in response to certain organic acids, delay the gastric emptying rate. The relative importance of enzyme inhibition and inhibition of the rate of gastric emptying is not clear, and conclusions regarding the physiological mechanisms by which, for example, sodium propionate lowers glycemia vary [65,171]; however, available data give rise to an increased interest in sourdough baking and other traditional fermentation processes.

11.4.3.5 Miscellaneous

In studies by Brand et al. [59], potato chips evoked a lower postprandial glycemia than would be anticipated from measurement of the in vitro rate of starch hydrolysis, indicating that the higher fat content in this product caused a delay in the rate of gastric emptying. Available data from the coingestion of fat with starchy meals suggest that different types of fat may behave differently in this respect. Thus, whereas butter either had no effect on acute glucose response [178] or delayed the glucose response [179], unsaturated oils significantly blunted glycemia in healthy volunteers [179]. The importance of the concurrent ingestion of fat and other meal constituents at more moderate levels,
as well as the effects of fat content or fatty acid pattern in different food items, should be studied in more detail.

11.4.4 In Vitro Prediction of Glycemic Index

The rate of amylolysis was early identified as an important determinate of postprandial glycemia [5]. Several enzymic in vitro procedures have therefore been suggested for the evaluation of the rate of in vivo starch digestion. Enzyme incubation is either performed with amylases only [102,169] or in combination with proteolytic enzymes [103,152,154]. The methods also differ — incubations are performed either unrestricted or restricted (that is, employing dialysis). The advantage of dialysis is that potential diffusion resistance caused by the viscosity of the digesta may be reflected. A good correlation has been obtained between the in vitro rate of digestion or the rate of release of starch hydrolysis products and glycemic responses in several studies [5,25,41,46,72], whereas no correlation was obtained in the case of legume products [180]. Possible causes of the lack of predictability are the presence of food factors that affect the rate of gastric emptying, such as certain dietary fiber (particularly if using unrestricted incubation procedures), organic acids, or fat. The mechanical disintegration of the sample prior to enzyme incubation may also affect the rate of amylolysis [101]. An in vitro procedure based on the enzyme incubation of chewed samples was therefore suggested [104]. This method has been shown to predict the GIs for a large number of starchy products, including legumes.

11.5 Resistant Starch

11.5.1 Resistant Starch Intake

We still lack sufficient data to predict accurately the amount of RS ingested in a Western diet. The tedious in vivo procedures do not provide a useful tool for characterization of the RS content in various foods. Although several in vitro procedures for RS determination have been suggested, their accuracy must await a more elaborate comparison with in vivo data. Based on current knowledge, however, it can be concluded that starch in ordinary foods enters the large bowel of healthy subjects in important amounts. A rough estimation suggests a daily intake of between 3 and 20 g [21,181,182], based on an average starch intake of 200 g in Western societies [33].

11.5.2 Sources of Resistant Starch in the Diet

Potential forms of resistant starch in the diet are presented in Table 11.4, and the RS contents of some foods are listed in Table 11.5. Major mechanisms for the delivery of RS to the large bowel in the case of heat-treated food items...
TABLE 11.4
Potential Forms of Resistant Starch in the Diet

- Raw starch granules (B-type)
- Retrograded starch
- Physically inaccessible starch (enclosed in cell or tissue structures)
- Chemically modified starch
- Thermally modified starch
- Amylose–lipid complexes (?)

are retrogradation and botanical entrapment of the starch substrate. In the raw state, certain B-type starches (potato, banana) may also escape digestion and absorption in the upper gut [183]. Enzyme resistance due to botanical entrapment is likely to be of particular importance in cereal products based on intact kernels and legumes, whereas retrogradation appears to be affected by the amylose/amylopectin ratio as well as by certain processing conditions. Processing conditions favoring RS formation, by retrogradation of the amylose in particular, include repeated cycles of heating and cooling [147,150]. In general, the more ordered the food form or the higher the crystallinity of the starch moiety, the higher the probability for the presence of RS.

Available data from human experimental models with realistic food items are scarce. Results from balance experiments in patients who have undergone ileostomy indicate RS figures of about 6% RS (starch basis) for baked white beans [21], compared with about 20% for a purée based on a precooked white bean flour with remaining cells [20]. As judged from H₂ measurements in expired air following ingestion of various legumes, RS ranged from about 13% in red kidney beans and lentils (available carbohydrate basis) to 20% in canned romano beans and navy beans [25,184]. Some data are also available from animal models. Measurements of ileal digestibility in the rat predicted RS figures around 10% in precooked lentil or red kidney bean flour with cell-enclosed starch [185] as well as in processed cow peas [186]. Levels were somewhat higher (11 to 15%) in the case of canned peas, lima beans, or kidney beans [187].

The ileostomy model gave a figure for RS contents in kernel-based cereal products close to 6% (starch basis) in pearled barley and less (about 3%) in rice [21]. The amount of starch recovered in the ileostomy effluent was considerably lower with ground rice (<1%, starch basis), demonstrating an impact of the botanical structure. The RS figure for intact rice is similar to that estimated indirectly from H₂ measurements [25]. Predicted values with this technique ranged from 9 to 13% in the case of pearled barley and pumpernickel bread products [25]. In contrast, although discovered early in bread, data from patients with ileostomies indicate low levels of RS in flour-
based products (less than 3%, starch basis) [188,189], mainly in the form of retrograded amylose. Only small differences were noted in RS recovery from sodium-bicarbonate-leavened bread, whether baked from finely or coarsely milled wheat flour [118]; however, RS data estimated from breath H\textsubscript{2} measurements gave significantly higher levels in pumpernickel bread than in flour-based products, again emphasizing the importance of an intact botanical tissue [25]. Due to the significance of bread products in the diet, bread is probably a quantitatively important source for RS.

Ileostomy data with corn flakes suggest an RS content of 3 to 4% (starch basis) [21,187]. This is close to the figure reported for corn arepas passing the rat small intestine [31]. Considerably higher amounts were passed to the human small intestine in the case of bread with added high-amylose corn starch — 24% (starch basis) (Jane Muir, pers. comm.) [29]. Approximately 30% of total starch reached the rat hind-gut in the case of arepas made from high-amylose corn (70% amylose) [31]. These figures are definitely in the upper range reported for composite ready-to-eat food items subjected to common cooking conditions. The nature of RS in arepa products is probably retrogradation of

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**TABLE 11.5**

<table>
<thead>
<tr>
<th>Product</th>
<th>Percent (%) (Total Starch or Available Carbohydrate Basis)</th>
<th>Refs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ground rice</td>
<td>1</td>
<td>21</td>
</tr>
<tr>
<td>Flour-based bread</td>
<td>&lt;3–8</td>
<td>25,188,189</td>
</tr>
<tr>
<td>Corn flour bread (arepas)</td>
<td>4</td>
<td>31</td>
</tr>
<tr>
<td>Corn flakes</td>
<td>3–4</td>
<td>21,188</td>
</tr>
<tr>
<td>Potatoes, freshly cooked</td>
<td>3</td>
<td>190</td>
</tr>
<tr>
<td>Rice</td>
<td>3–5</td>
<td>21,25</td>
</tr>
<tr>
<td>Pearled barley</td>
<td>6–13</td>
<td>21,25</td>
</tr>
<tr>
<td>Pumpernickel bread</td>
<td>9–11</td>
<td>25</td>
</tr>
<tr>
<td>Potatoes, cooled/reheated</td>
<td>8</td>
<td>190</td>
</tr>
<tr>
<td>Potatoes, cooled</td>
<td>12</td>
<td>190</td>
</tr>
<tr>
<td>Canned infant pea/potato product</td>
<td>18</td>
<td>30</td>
</tr>
<tr>
<td>Legume products</td>
<td>6–20</td>
<td>20,21,25,184,185,187</td>
</tr>
<tr>
<td>Bread made with high-amylose corn starch</td>
<td>24</td>
<td>29; J. Muir, pers. comm.</td>
</tr>
<tr>
<td>High-amylose corn flour bread (arepas)</td>
<td>30</td>
<td>31</td>
</tr>
<tr>
<td>Drum-dried hydroxypropylated potato starch</td>
<td>50</td>
<td>193</td>
</tr>
</tbody>
</table>

* Range in resistant starch with different *in vivo* methods. See text for details.
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amylose, although incomplete swelling of starch granules cannot be excluded in the case of high-amylose corn. A finding in support of retrogradation is the differences observed in fermentability characteristics for RS in high-amylose arepas prepared by repeated heat-treatment and RS in the form of raw high-amylose corn starch (see Section 11.5.4.2.). A somewhat higher in vitro RS content was also observed in rice [140] and barley porridges [92] in products based on high-amylose rather than normal genotypes. High RS levels were also present in autoclaved infant purées based on peas and potatoes, as judged from balance experiments in antibiotic-treated rats — about 18% (starch basis) [30].

Potato products represent another potentially important source of RS in the diet. Data from experiments in patients who have undergone ileostomy suggest low figures in freshly cooked potatoes — about 3% of ingested starch [190]. Significantly higher levels were present following cooling (12%). A similar effect on RS yield following cooling of potatoes was also reported in in vitro studies [191]. Reheating of the cooled potatoes significantly decreased the amount of RS recovered in the ileostomy effluent, from 12% to 8% [190], suggesting that retrogradation of amylpectin, not amylose, was the major cause of the formation. Evidence suggests the involvement of amylpectin in the case of RS from potato starch. Whereas RS formation in autoclaved cereal starches was explained in terms of amylose retrogradation, retrogradation of amylpectin was suggested for the case of potato starch [192].

Other minor components such as chemically modified starch (CMS) [193] or thermically modified starch [194] can also be expected to contribute to the total amount of RS in a diet. Thus, as judged from measurements of ileal digestibility in the rat, gelatinized potato starch with bulky substituents such as hydroxypropylated starch was poorly digested by amylases, with about 50% of the ingested amount being passed to the hind-gut [193]. Although not a significant form of RS, CMS is present in small amounts in many prefabricated products, including infant food. According to calculations in 1988 [195], CMS intake in infants from 2 to 12 months of age ranged between 1 and 17 g daily. The digestibility and colonic fate of these derivatives should be studied in more detail.

Although they reduce the rate of amylolysis of the amylose component, it is not known if inclusion complexes with lipids add to the RS contents of foods. Analysis of the starch contents in the small intestine of rats intubated with either amylose or amylose–lycokithine complex has indicated a high small-intestine digestibility [156]. No data are available based on more quantitative in vivo techniques. As for the contribution of amylose–lipid complexes to RS in processed food, autoclaving of starch in the presence of monoglycerides was found to reduce formation compared with no addition [196], suggesting that the inclusion complex disturbs the crystallinity of the retrograded amylose. The RS fraction in these experiments was isolated using treatment with thermostable amylases. Incubation at such elevated temperatures can be
expected to dissociate the complex and thus render it more susceptible to enzymic hydrolysis than when it is exposed to amylases at physiological conditions. As a result, no conclusions can be made at the present time regarding the possible contribution of amylase lipid complexes to RS formation; however, measurements of ileal digestibility in humans indicated 21% RS (total starch basis) for autoclaved high-amylose starch added with monoglycerides, compared with greater than 50% in extrusion-cooked and cooled starch, containing mainly retrograded amylose [197].

Certain raw B-type starches may contain appreciable portions of RS. Considerable amounts — approximately 84% raw potato starch [198] and 69 to 76% banana starch (starch basis) — have been reported to pass the human small intestine undigested [198,199]. As judged from experiments in rats, approximately 30% was RS in raw potato starch and only minute amounts were present in raw pea starch (C-type) [200]. Raw cereal starches (A-type starches) are generally considered to be more or less completely digested and absorbed in the small intestine. Information regarding RS contents in various raw starches is interesting from a mechanistic rather than a nutritional point of view, as most foods are consumed following heat treatment; however, the presence of starch in unripe bananas may significantly contribute to RS intake in diets rich in plantain bananas. Apples can also be expected to contain some starch in the unripe state (Kåre Larsson, pers. comm.). The potential contribution of RS from temperate fruits is not known.

Whether antinutrients such as polyphenols, phytic acid, or lectins may affect the RS content of foods remains to be established. The addition of such substances (tannic acid, catechin, sodium phytate) to experimental diets has been shown to reduce the ileal digestibility of protein and fat in the rat but to have no influence on starch digestibility in chicks when added to amounts up to 24 g/kg test diet [202]. In contrast, as judged from breath H\textsubscript{2} measurements in human subjects, bread made from undephytinized navy bean flour contained higher amounts of RS (17%) than a corresponding bread product from dephytinized flour (9%) [169]. It should be noted, however, that the dephytinization process involved incubation with phytase at 55°C for 6 hours followed by freeze-drying and milling. It is possible that the thermal and mechanical treatments may have increased the small-intestine digestibility of the starch in this product.

11.5.3 In Vitro Methods for Resistant Starch Analysis

The different nutritional characteristics of starch, whether digested and absorbed at various rates in the upper gastrointestinal tract, thus evoking low or high postprandial blood glucose peaks, or passed undigested to the large bowel, have emphasized the need for a nutritional classification of starch. A classification into “readily digestible,” “slowly digestible,” and “resistant”
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starch was suggested by Englyst et al. [203], based on the availability for enzymic digestion in vitro. The distinction between starch that is absorbed in the small intestine and resistant starch, however, is not clear from an analytical point of view. The enzyme resistance of RS is therefore not absolute, and the amount escaping digestion in vivo may depend on extrinsic factors such as the extent of chewing, the rate of orocecal transit, or the amount of starch ingested [204,205]. The incubation conditions may likewise affect in vitro RS yield [203,206].

Several enzymic methods for the quantification of RS levels in foods have been presented. All these methods consist of enzymic removal of the potentially available starch fraction and subsequent quantification of the remaining “resistant” fraction. In the Berry method [147], RS is defined as the starch that survives exhaustive digestion with amylolytic enzymes (α-amylase, pullulanase). The ground sample is analyzed as is (without additional heat treatment), and enzyme resistance due to the presence of raw granules might thus be reflected. The total RS residue is quantified following treatment with KOH, which solubilizes retrograded or ungelatinized starch and also disrupts the integrity of intact cell walls [154]. Other methods include the analysis of total starch remnants in an enzymic gravimetric dietary fiber residue, obtained following boiling of the milled sample with a thermostable α-amylase (Termamyl®) and subsequent incubation with pepsin and pancreatin [27]. The RS fraction mainly consists of retrograded amylose, as neither botanically encapsulated starch nor raw starch granules are recovered. A measure of a similar retrograded amylose fraction can also be obtained by subtracting the potentially available starch analyzed without alkali treatment from the total starch analyzed following alkali solubilization and initial boiling with Termamyl [154]. As judged from current knowledge, retrograded starch appears to be a major RS fraction in many foods [203]; however, although these analytical procedures may provide a relevant ranking of RS contents for some products, such as fully gelatinized flour-based products [30,31,185], they are all inadequate predictors of in vivo RS contents in products in which the botanical or physical structure limits enzymic accessibility. Also, apart from gelatinizing the starch, incubation with Termamyl at elevated temperatures may to some extent also dissociate firmly retrograded amylose, thus underestimating in vivo resistance [206]. This might be the case also with amylose–lipid complexes.

To avoid these limitations, attempts have been made to mimic the digestive phase in vivo. The most elaborate is probably the method suggested by Englyst et al. [203], which allows parallel estimation of enzyme resistance due to raw starch, retrograded starch, and botanically encapsulated starch. Although it avoids incubation temperatures that may affect the solubility of the starch, the enzyme incubation is still performed on mechanically disintegrated samples that may affect RS yield in comparison with the in vivo situation; however, with the products studied so far, good agreements have been obtained with
the ileostomy model [203]. An in vitro RS procedure based on enzyme incubation of chewed samples was recently developed [191]. Results of this method also compare favorably with RS estimated from starch recovery in ileostomy effluents [21].

The most studied RS fractions discussed above—botanically encapsulated starch, raw B-type starch, and retrograded starch—are commonly referred to as RS1, RS2, and RS3, respectively, according to the terminology introduced by Englyst and coworkers. Other potential RS candidates (e.g., certain CMS [193] or thermically modified starches [194]) are likely to be underestimated by all of the methods described here for RS determination. As a result, indigestible starch remnants following amylase incubation of gelatinized hydroxypropylated starch were found to have a high degree of substitution, and glucose residues adjacent to substituted glucose molecules were not hydrolyzed by analytical amylases. An additional problem is that, even when acid hydrolysis is used, liberated hydroxypropylated glucose monomers could not be quantified by conventional analysis [207].

The reliability of different in vitro procedures for RS determination is currently a topic of great interest. Balance experiments in patients who have undergone ileostomy will provide necessary reference data to be compared with in vitro RS content obtained by different methods.

Some of the limitations discussed above are also present when animal models are used for RS quantification. Whereas phenomena linked to, for example, retrogradation or botanical entrapment at a cellular level may be reflected in, for example, rat experimental models, the potential obstruction of amylases due to the enclosure of starch in botanical tissues (e.g., grains) is not. This should be kept in mind when interpreting data from animal experiments. With flour-based, fully gelatinized material, RS as estimated from balance experiments in antibiotic-treated rats has shown results in good agreement with RS figures obtained from the analysis of starch remnants in an enzymic gravimetric dietary fiber residue [208].

### 11.5.4 Nutritional Effects of Resistant Starch

In the colon, RS constitutes a potentially available carbohydrate source for the colonic microflora. This fermentation is discussed here with relation to its beneficial effects on the metabolism and maintenance of colonic health.

#### 11.5.4.1 Colonic Formation of Short-Chain Fatty Acids

The colonic formation of resistant starch lowers colonic pH by increasing the content of various organic acids. The main acids produced are the so-called short-chain fatty acids (SCFAs) — acetic acid, propionic acid, and butyric acid [29]. Fermentation of indigestible carbohydrates, such as inulin, also leads to
the formation of lactic acid [209]. It is not known to what extent lactic acid is also generated from RS. According to Mathers [210], lactate production is probably a transient phenomenon occurring mainly in the initial stages of starch fermentation; however, others have indicated [211] that RS in the form of raw potato starch produces very acid feces but low SCFA concentrations. It has been suggested that RS fermentation produces organic acids apart from the SCFAs commonly referred to (acetic, propionic, and butyric acid) [29]; however, the production of such organic acids may vary with the type of RS. The decrease in rat cecal pH was more pronounced with raw potato starch than with amylo- maize starch, despite a lower cecal content of SCFAs in the potato starch [212].

The SCFAs formed are absorbed from the colonic lumen into the mucosa. Butyric acid is the preferred energy source of the colonocytes [213], and it suppresses tumor proliferation in vitro [214]. Acetate, propionate, and residual butyrate enter the portal blood and are transported to the liver. Propionate is increasingly being discussed in relation to its beneficial effects on glucose and lipid metabolism [171,215]. Most of the studies conducted so far have been with dietary supplemental propionate; however, in a recent study, rectal administration of propionate at levels that can be expected to be generated from colonic fermentation reduced total liver cholesterol in hyperinsulinemic rats [216].

It is still a matter of controversy if the reduction seen in serum cholesterol with certain viscous, and fermentable, dietary fiber components is due to increased fecal losses of bile acids or cholesterol caused by fiber entrapment or if colonic generation of propionic acid may contribute [183]. Although less pronounced than with guar gum, the addition of RS in the form of heat-treated high-amylose maize starch to rat experimental diets was found to lower serum total cholesterol following 4 weeks of administration [217]. In experiments in patients who have undergone ileostomy, the addition of a similar RS fraction to the diet reduced the ileal loss of cholic acid. The cause for this is not known, but the phenomenon does not favor a mechanism related to the drainage of sterols from the enterohepatic circulation by binding to RS. The lowering of serum triglyceride levels found by Sacquet et al. [218] in rats fed amylo maize starch could only partly be accounted for by increased fecal sterol excretion; thus, it is possible that the lowering of plasma cholesterol and triglyceride levels noted in rat experiments is mediated by colonically derived SCFAs. Other types of RS fractions evaluated in this context include cyclodextrins. According to Suzuki and Sato [219], the addition of cyclodextrins containing seven glucose residues improved parameters related to lipid metabolism in rats.

Of interest in relation to colonic health and the RS contents of foods are studies suggesting that avid digestion and absorption of starch in the small intestine may be associated with colon polyps [220], although results by Mathers did not support this theory [221]. In light of the role of butyrate as an energy source for the colonic mucosal cells, the ability of starch to yield butyrate upon fermentation has therefore attracted special attention.
According to Englyst et al. [222], *in vitro* fermentation with human fecal inoculums indicated a significantly higher butyrate proportion with starch (29%) than with certain dietary fiber components (2 to 8%) as substrate. A similar conclusion was drawn from *in vitro* fermentability studies with corn starch vs. cabbage fiber [223]. In experiments in rats, however, the proportion of butyrate following fermentation of starch in the hind-gut was in the mid-range (~10%) of that reported for other indigestible carbohydrates, including oligosaccharides (4 to 22%) [224].

A few studies have examined potential differences in SCFA patterns from various RS sources. *In vitro* incubation with human or rat feces indicated no differences between RS remnants prepared from autoclaved maize or pea starch, respectively, by enzymic incubation and centrifugation of the gelled starches [225]. The butyrate proportion was between 5 and 8% of total SCFA generated. A somewhat higher proportion (between 13 and 23%) was reported in a similar *in vitro* study with raw corn starch [223]. Whether these differences reflect differences in properties of RS in the form of retrograded vs. raw starch cannot be deduced; however, experiments in rats have indicated that the distribution of SCFA in the hind-gut may differ depending on the source of RS. The distribution of acetate–propionate–butyrate was 54:33:13 in the hind-gut of animals fed high-amyllose starch compared with 81:18:1 in the case of potato starch [226]. In contrast, the distribution of SCFA in rat cecum was similar, or about 70:15:10 in animals fed raw potato starch and retrograded wheat starch, respectively [200].

No conclusions can be made regarding whether starch is a particularly good source of butyrate generation in the human colon or if there are nutritionally significant differences in fermentability pattern between various forms of RS in common foods. Nonetheless, in humans, the addition of an α-glucosidase inhibitor (acarbose) to the diet tended to increase specifically the yield of butyrate in feces, suggesting that an increased delivery of starch to the colon was accompanied by a higher butyrate concentration in the distal colon/feces [227]. The site for butyrate generation may also be of importance in relation to colonic tumor development [29]. A more distal production of butyrate might be more advantageous, because most colonic tumors develop at this site. As a consequence less readily fermentable RS substrates might also be beneficial. Of interest in this context are results from *in vitro* fermentability studies with rat or human feces [225]. The rate of fermentation of a retrograded amylase fractions from autoclaved pea starch was significantly slower than with a corresponding amylase fraction from autoclaved maize starch.

Apart from the yield or site of butyrate formation, an acidic environment in the colon may have implications. It has been suggested that a low luminal pH may be protective against the effect of carcinogenic bacterial metabolites (e.g., ammonia and secondary bile acids) [228].
11.5.4.2 Fecal Bulking

Although it remains to be established if the molar distribution of SCFA differs between RS sources, the total fermentability may vary; thus, retrograded starch in wheat bread, autoclaved wheat starch suspensions, or canned infant pea/potato purées was readily fermented in the rat hind-gut [30,150,229]. In contrast, RS in high-amylose corn bread arepas [31] or hydroxy-propylated potato starch [193] was also resistant toward bacterial amylases in the rat hind-gut, with less than 10% being fermented. The low fermentability in the case of the high-amylose corn product is in contrast to the results of Andrieux et al. [229] for raw high-amylose corn starch, which was fermented to a high degree in the rat hind-gut. This may suggest that the RS in the high-amylose arepas consisted of a fraction other than ungelatinized starch. Differences in hind-gut fermentabilities of RS fractions have also been observed in legume products processed under similar conditions [185]; thus, RS in a red kidney bean product was fermented to 85% vs. only 40% in a corresponding product from green lentils.

Whereas a high fermentability will favor colonic SCFA production, a low fermentability will instead have more profound effects on fecal bulk. In humans, laxative effects have been reported following ingestion of raw potato or banana starch and retrograded wheat or maize starch, although this effect was less prominent than with a corresponding amount of wheat bran [231]. According to Shetty and Kurpad [232], RS present in a corn starch gruel significantly increased fecal bulk in humans, despite the fact that no starch could be detected in feces. In analogy with the bulking effects noted with fermentable types of dietary fiber components, this fecal bulking is probably due to an increased bacterial mass.

11.5.4.3 Resistant Starch in Relation to the Glycemic Index and Metabolic Responses

The resistant starch content of foods may influence the GI and glucose metabolism in humans in several ways. First, only the non-RS fraction of ingested starch has the potential of increasing postprandial blood glucose response. Because most current methods for starch analysis may partly include RS, the available starch content in test products high in RS is likely to be lower than predicted. Second, depending on the cause of enzymic resistance or mechanism for RS formation, the extent of digestion may be linked to the overall rate of starch digestion. Third, the short-chain fatty acids produced during colonic fermentation of RS may enhance glucose tolerance, at least at the subsequent meal or in a long-term perspective.

A relation has been reported to exist between the GI and RS content of foods [106], and some of the factors that affect the postprandial glycemia
response to starch are known to also affect the total digestibility in the small intestine (e.g., the crystallinity in certain raw starches or botanical entrapment of starch). At least theoretically, the lowered glycemia response seen with certain products could be because a lower amount of available starch was actually ingested; however, as judged from our current knowledge regarding the RS content of various foods, the differences in available starch *per se* are in general too small to account for the large differences noted in metabolic responses. This is also the conclusion drawn in various papers addressing this topic [106,151,169,233]. Due to the dynamic regulation of the blood glucose level, within certain limits, it appears plausible that the rate of delivery of glucose to the blood, and in particular the early rate, is a more important determinant of the course of postprandial glycemia than the total amount of available starch ingested.

With regard to the second possibility, substantial amounts of RS may be present in certain bread products (about 8%) without affecting the *in vitro* rate of starch digestion in the case of the “available” starch fraction [64]. In contrast, the formation of RS in high-amylose arepa products (about 30%) was accompanied by a lowered rate of enzymic digestion of the non-RS fraction, compared with corresponding arepas made from common corn flour [31]. The differences in glycemic responses to these arepa products also persisted when the products were compared on a potentially available starch basis. In fact, no differences were noted in glycemic response to the high-amylose arepas, whether included in a test meal on the basis of total or potentially available starch basis. This suggests that, even at high levels of RS, a lowered rate of enzymic hydrolysis of the non-RS fraction, not a lowered amount of available starch, was the major determinant of the lowered glycemia observed.

Long-term experiments with low-GI diets have indicated beneficial effects on not only glucose metabolism but also blood lipids. These metabolic effects are commonly attributed to a lowered rate of starch digestion and absorption in the upper gut. A smaller postprandial increase in insulin may reduce hepatic lipogenesis [81,234] and suppress free fatty acid levels, thus increasing insulin sensitivity [81]. Many “slow-release” starchy products also contain important amounts of RS. The colonic generation of SCFAs from fermentation was recently suggested as a mechanism for the improvement of glucose metabolism [29]. An indication that this might be the case is the finding that an increased delivery of fermentable carbohydrates to the colon was associated with an increased glucose tolerance 12 hours later [82]. The suggested key event was a lowering of serum-free fatty acid levels or a reduced hepatic metabolic response to a subsequent meal [233]. Such a “second meal” effect was noted on insulin response at lunch following a breakfast with pasta compared with a corresponding breakfast with white bread [91]. The pasta and bread, however, could be expected to contain similar amounts of RS but differed in their rate of release of starch hydrolysis products during *in vitro* incubation [51].
In conclusion, whereas the presence of RS *per se* is probably not the cause for differences in GI, RS content may still be of importance in relation to glucose metabolism. More studies are needed to establish the relative importance of the fate of the potentially available starch fraction in the upper gut, compared with events of the RS fraction in the colon in relation to long-term metabolic effects of low-GI starchy foods. Both the postprandial blood glucose and insulin responses and the colonic formation of short-chain fatty acids may possibly be of importance.

### 11.5.4.4 Miscellaneous

Other physiological effects of RS mediated in the gastrointestinal tract include a lowering of the excretion of sterols. RS in autoclaved high-amylose maize starch significantly reduced the ileal excretion of colic acid in proctocolectomized subjects compared with a reference maize starch diet with less RS [235]. No effects on the availability of minerals and other nutrients were noted. Due to the potential microbial metabolism of bile acids into compounds with carcinogenic potential [228], RS fractions, by reducing the colonic entry of bile acids, might have a protective role in colonic cancer. Also of interest in this respect are the reduction noted in cecal bacterial biotransformation activities (β-glucosidase and β-glucuronidase) and apparent ammonia production in rats fed raw potato or amylomaize starch, respectively [226]. Concerning effects on the intestinal mucosa, RS appears to enhance cell proliferation in the ileum [236] and distal colon [211]. The physiological impact of these changes in mucosal morphology should be evaluated. Some data are also available from the patent literature [237], suggesting that RS in the form of thermically modified starch produced by heat-treatment at dry conditions may have hypotensive effects in humans.

At elevated levels of intake, RS may produce abdominal discomfort [238]. In general, however, the metabolic events linked to colonic fermentation or fecal bulking of RS are probably advantageous in the diet for adults. This is not necessarily the case in the infant diet, and the presence of considerable amounts of RS in infant purées (18%, total starch basis) should be recognized, as they add to the total load of indigestible carbohydrates [30]. Other connections between RS and the total load of indigestible carbohydrates should also be considered; thus, it has been suggested that dietary fiber increases the ileal loss of starch in children [239] as well as in adults [240].

### 11.6 SUMMARY

Starch is not only the quantitatively most important source of available carbohydrates but also an important source of indigestible carbohydrates in the diet. The different GI characteristics of various foods have interesting implications
with regard to both the prevention and management of metabolic disease. A growing body of evidence also suggests the important nutritional features of the RS fraction. RS might possibly contribute to the advantageous effects previously assigned solely to dietary fiber. Current knowledge definitely indicates a great challenge for plant breeders and the food industry to optimize the nutritional properties of starch in foods; however, in order to elucidate fully the nutritional potential of differences in GI or RS contents of food, more long-term studies are needed with test diets well characterized as to these parameters.

REFERENCES


Carbohydrates in Food


