Functionalizing Carbohydrates *for* Food Applications

Texturizing and Bioactive/Flavor Delivery Systems

Edited by

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Preface

CARBOHYDRATES are the most abundant class of organic compounds. They are essential for human health because they supply short-term (e.g., mono- and disaccharides) and intermediate-term energy (e.g., starches). Carbohydrates serve as the primary energy source. They are also important in the diet due to specific functions they perform in the human body such as eliminating or minimizing ketosis, breakdown of body protein, loss of cations and dehydration. In addition, carbohydrates are essential because they are precursors to carbohydrate derivatives that are actively involved in human metabolic processes such as fertilization, immune systems, development of diseases, and blood clotting. Carbohydrates are also critical structural components of plants.

As important as carbohydrates are in humans and plants, they are equally important functional ingredients in food products. Due to their ubiquitousness, versatility and unique structures, carbohydrates are prime materials and will remain major candidates for functional food ingredients. Celluloses, starches and gum polysaccharides comprise the largest group of biomolecules. Because they are readily available, renewable and sustainable, they are popularly used as raw materials in preparation of much needed functional food ingredients. Two very important functions of carbohydrates in prepared foods are as food texturizers and as delivery matrices for flavors and bioactives. Carbohydrates have the ability to positively alter and improve rheological properties of food products to make them more desirable and to prolong their shelf life. They are also able to function exceptionally as emulsifiers and en-

capsulators to protect and preserve flavors and bioactives that no other biomolecules and other substances are able to do. It is the goal of this book to provide fundamental, practical information about carbohydrates and their utilization as texturizing systems in foods and as delivery matrices for flavors and bioactives. Both substances are very important: the first for food product desirability, quality, and acceptability, and the second for health and wellness, which has become the focus of consumers for the past 10 years. This book brings together carbohydrate experts in the fields of carbohydrate chemistry, starch and polysaccharide rheology, flavor and bioactive encapsulation and delivery systems. This book starts with a chapter on essentials of carbohydrate chemistry written by a world renowned professor and carbohydrate chemist to provide the reader the necessary background on carbohydrates and/or review materials on carbohydrates so it will not be necessary for the reader to consult another book on the subject while reading the other chapters. The next two chapters are on texturizing systems using starches and polysaccharide gums, written by two experts in the field who have more than 35 years of combined experience with these groups of versatile food ingredients. The next chapter on flavor delivery systems is written by a professor whose expertise in this area has made his name synonymous with flavor encapsulation. He is the most well-published professor in this area, not only in the United States but also throughout the world. The last three chapters deal with delivery systems for bioactives, processing methods used in encapsulation and emulsion technologies, and analytical methods. We hope these chapters will provide ideas in assessing carbohydrates and polysaccharides that will be useful in correlating structure with function, and help readers design their own processes and set up their own analytical tools for the evaluation of encapsulated products and emulsions. This book is intended to provide a central source of theoretical information and practical knowledge both for the novice and those already working in these areas.

Each chapter has been developed in a comprehensive manner, so that each can stand on its own. Due to the scope and nature of each chapter, overlap of some topics cannot be avoided. These overlaps are necessary so that each chapter stands on its own.

I sincerely and greatly appreciate the work of the professors, researchers/scientists, and investigators who have worked very hard to understand the topics covered in this book and who shared their knowledge and findings with the rest of the world. They continue to work passionately on these areas to keep the interests and advancement of science

in these fields alive and strong. Special thanks also goes to DEStech Publications, Inc. and their staff, especially to Dr. Joseph L. Eckenrode, Editorial Director, for his encouragement, patience and whole-hearted support of this book project. Special thanks to Saulo Embuscado for preparing some excellent figures in this book.

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Essentials of Carbohydrate Chemistry

JAMES N. BeMILLER, Ph.D.

1.1. OVERVIEW

1.1.1. Background and Historical Perspective

MORE extensive descriptions of the essentials of carbohydrate chemistry important to food applications have been prepared by Tomasik (2004), Izydorczyk (2005), BeMiller (2007), and Wrolstad (2012). Other broader presentations of carbohydrate chemistry (not primarily related to food applications) have been prepared by Pigman and Horton (1970, 1972, 1980), Lehmann (1998), Robyt (1998), Lindhorst (2007), and others. Included here are highlights of chemical and physical properties and primary applications (but, with a few exceptions, not health attributes) of major carbohydrates used as food ingredients. Emphasized are those carbohydrates used in the greatest amounts or with unique properties.

1.2. NATURE OF CARBOHYDRATES

Carbohydrates are polyhydroxylated compounds that are, for the most part, at least partially water soluble, and if not, water sorbing. They are widely used as ingredients in a broad range of food products

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primarily because of the functionalities they impart (Lai and Lii, 2004). These functionalities may include thickening, control of water activity, and provision of specific rheological attributes, emulsion and suspension stabilization, encapsulation, gel formation and characteristics, mouthfeel, humectancy, glass formation and transitions, crispness, and sometimes sweetness.

Most carbohydrates in nature (in plants) are in the form of polysaccharides, which means they are polymers of simple sugars/monosaccharides (Section 1.4). Lesser amounts, by mass, in nature are in the form of oligosaccharides, which are short chains of monosaccharides (Section 1.4). Very little carbohydrate in nature is present as monosaccharides (Section 1.3). However, mono- and oligosaccharides, primarily from starches, but also other polysaccharides, are produced industrially via depolymerization of polysaccharides.

1.3. MONOSACCHARIDES

Monosaccharides (BeMiller, 2007, pp. 1–68) are carbohydrate molecules that cannot be broken down by hydrolysis to smaller carbohydrate molecules. Also called "simple sugars", they are chiral, polyhydroxylated aldehydes (aldoses) or ketones (ketoses) and contain from 3 to 9 carbon atoms. Most are 6-carbon-atom aldehydo sugars (aldohexoses), one of which, D-glucose, known commercially as dextrose, is used here as an example.

The most abundant carbohydrate, if all its combined forms are included, is the aldohexose D-glucose. Its acylic (non-cyclic) structure is shown in Figure 1.1.

However, neither D-glucose nor any other monosaccharide occurs in

$$
\begin{array}{c}
CHO \\
| \\
HCOH \\
| \\
HOCH \\
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HCOH \\
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HCOH \\
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CH_2OH
\end{array}
$$
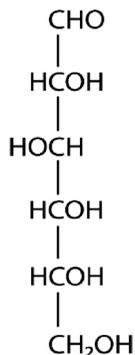
FIGURE 1.1. D-Glucose in its acyclic structure.

FIGURE 1.2. *D-Glucose in abbreviated (ring carbon and attached hydrogen atoms omitted) α- (right-hand) and β- (left-hand) pyranose ring forms.*

the acylic open-chain structure, whether in a combined form or as a free sugar, other than in the very small percentage of cases when the free sugar is in solution. Rather the sugars are in ring forms, primarily in a six-membered ring called a pyranose ring, which is formed (in aldoses) when the aldehydo group reacts with the hydroxyl group on the fifth carbon atom to form a cyclic hemiacetal, creating a new chiral carbon atom, which can occur in two different configurations. These two configurations (Figure 1.2) are designated alpha and beta. In the structures pictured, the carbon atoms at the corners of the rings and the hydrogen atoms attached to them are omitted for clarity. Pyranose rings also occur in different shapes, known as ring conformations.

The structure of the most abundant ketose (D-fructose, a ketohexose) is given Section 1.4.1.1).

Monosaccharides also occur in oligo- and polysaccharides in a five-membered ring known as a furanose ring. Five-carbon-atom aldoses (aldopentoses) occur in hemicelluoses (D-xylose in the pyranose ring form and L-arabinose in both pyranose and furanose ring forms). Other monosaccharides are components of the polysaccharides known as food gums or hydrocolloids (Section 1.4.3.3).

Aldoses are known as reducing sugars because they can effect reduction of certain ions and compounds, during which process the aldehydo group of the aldose is oxidized to a carboxyl group (Section 1.5.2).

In food products, mono- and disaccharides can crystallize and/or form glasses (Jouppila, 2006). All impart colligative properties such as reduction in water activity, boiling point elevation, and freezing point depression.

1.4. CARBOHYDRATES CONTAINING GLYCOSIDIC LINKAGES

Most naturally occurring carbohydrates and most of those used as food ingredients, whatever the source, are compounds consisting of

monsaccharide units joined together in what are known as glycosidic bonds, a glycosidic bond being an acetal group formed from the hemiacetal hydroxyl group of a monosaccharide unit in one of the ring forms (pyranose or furnanose) and a hydroxyl group on another monosaccharide unit. The number of units joined together (the degree of polymerization, DP) ranges from two to more than 10^7. Those compounds with DPs of from 2 to 20 are called oligosaccharides (BeMiller, 2007, pp. 69–92); those with DPs of > 20 are called polysaccharides (BeMiller, 2007, pp. 93–117). In the majority of cases, the monosaccharide units are joined together in a head-to-tail fashion, and since most are composed of aldosyl units, one end (called the reducing end) contains a reducing (aldehyde or hemiacetal) group. Because each constituent monosaccharide unit contains more than one hydroxyl group, oligo-and polysaccharides may be branched. Branched structures will have more than one, usually many, non-reducing ends.

Other than sucrose, the primary sources of di- and higher oligosaccharides in foods are syrups, syrup solids, and maltodextrins produced by the hydrolysis of starch (Section 1.8.1) and milk (lactose) (Section 1.4.1.2).

1.4.1. Disaccharides

Disaccharides are compounds containing two conjoint monosaccharide units. Two naturally occurring disaccharides (sucrose and lactose) and one produced by the hydrolysis of starch (maltose) predominate. Disaccharides generally have the same properties as monosaccharides (Section 1.3).

1.4.1.1. Sucrose

One of the two monosaccharide units that make up the disaccharide sucrose is D-fructose (Figure 1.3), a ketohexose. In solution, D-fructose occurs primarily in the pyranose ring form, but in sucrose, it is present as β-D-fructofuranose (the lower unit of Figure 1.4). Crystalline fructose is made commercially by isomerization of D-glucose obtained from starch (Section 1.8.1).

Sucrose (Pennington and Baker, 1990; Mathouthi and Reiser, 1995; BeMiller, 2007, pp. 80–96), commonly called sugar or perhaps further categorized as cane sugar or beet sugar, is a unique carbohydrate. What makes it unique is that its constituent monosaccharide units are

CH₂OH
|
C=O
|
HOCH
|
HCOH
|
HCOH
|
CH₂OH
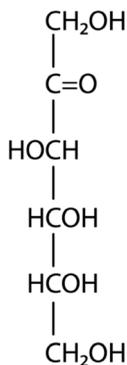
FIGURE 1.3. *D-Fructose in its acyclic structure.*

linked head-to-head, i.e., reducing end-to-reducing end, in a glycosidic bond, rather than the very much more common hemiacetal hydroxyl (reducing end) to hydroxyl group linkage. In the case of sucrose, α-D-glucopyranose is one of the monosaccharide units (the top unit in Figure 1.4) and β-D-fructofuranose is the other (the bottom unit in Figure 1.4). One of the hemiacetal groups provides the hydroxyl group to the other for formation of the acetal structure (a glycosidic bond). Because neither unit can open up to provide a carbonyl group (Figure 1.1), sucrose is a nonreducing sugar. However, because furanosides (compounds containing furanosyl groups, in a glycosidic linkage) undergo acid-catalyzed hydrolysis quite readily in the presence of heat and an acidic pH (even only mildly acidic), sucrose undergoes hydrolysis to release D-glucose and D-fructose. Therefore, there is some D-glucose
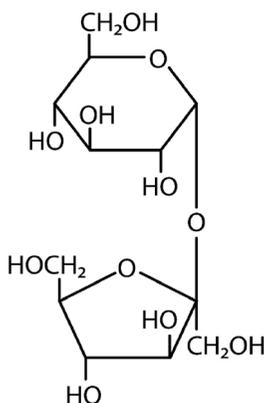
FIGURE 1.4. *Sucrose inan abbreviated (ring carbon and attached hydrogen atoms omitted) structure.*

present in many food products containing sucrose. In these cases, Maillard browning (Section 1.5.1) will create color and aroma.

Sucrose is of course employed as a sweetener, but it contributes many, many other functionalities as well, some of which cannot be contributed by other compounds (BeMiller, 2007; pp. 349–356). Some of its attributes are as follows. Sucrose, by binding water, delays gelatinization and pasting of starch in chemically leavened batters, such as cake batters, and prevents full hydration of gluten development in both yeast-leavened doughs and chemically leavened cake batters, thus improving volume and crumb texture. It is a creaming aid in shortened cake batters and cookie doughs, a whipping aid that stabilizes beaten egg foams in unshortened cakes, and it caramelizes in most baked products and is the base material in the production of carmel colors. By holding water, sucrose maintains moisture in many products and induces spread in some cookie doughs, crystallizes in various crystal sizes in confections (providing texture) and in certain baked products, to which it provides crispness. Sucrose forms highly supersaturated solutions and glasses in confection production, delays egg protein denaturation/coagulation during baking of unshortened cakes and custards, lowers the freezing point of ice cream and other frozen desserts, enhances and balances tomato, vinegar, and citrus flavors in barbecue sauces and salad dressings, balances the salty taste of brines and marinades, provides bulk and structure to icings, provides the necessary solids content for the preparation of standard jams, jellies, preserves, and marmalades (see under Section 1.4.3.3 Pectins) and reduces their water activity—i.e., acts as a preservative—and produces a stiffer, more stable meringue foam. Some, but not all, of these functions can be provided by glucose and/or high-fructose syrups (Section 1.8.1) and products such as sorbitol (Section 1.5.3.1). Especially difficult to mimic are those functionalities involving sucrose crystals and/or crystallization.

1.4.1.2. Lactose

Lactose (BeMiller, 2007, pp. 76–79) is a disaccharide found in milk (Figure 1.5). It contains a D-glucosyl unit at its reducing end (the right-hand unit in Figure 1.5). The D-glucosyl unit has attached to it, on the oxygen atom on carbon atom number 4, an α-D-galactopyranosyl unit, the two monosaccharide units being joined in a glycosidic bond, which because it is a –O– bond connecting carbon atom 1 (C1) of one glycosyl (monosaccharide) unit to C4 of another unit is known as a (1→4)- or
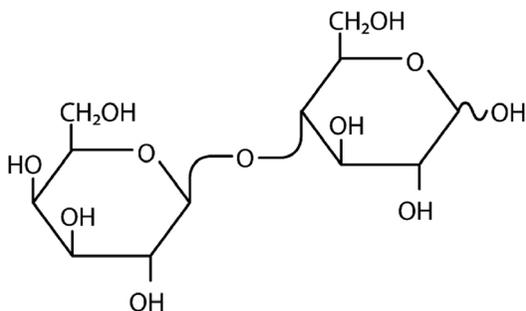
FIGURE 1.5. Lactose.

(1,4) linkage. In Figure 1.5, the D-glucosyl unit is shown in a hemiacetal form, i.e., in a pyranose ring form. Because the pyranose ring can readily open, generating an aldehyde group, lactose is a reducing sugar.

Lactose contributes body, with only about 40% of the sweetness of sucrose, to food products to which it is added; but it is used only to a small extent in certain ice cream products, icings, pie fillings, and confections.

1.4.1.3. Maltose

Maltose (Figure 1.6) is a product of hydrolysis of starch. It contains an α-D-glucopyranosyl unit connected to another D-glucosyl unit at C4 through a glycosidic bond. Because it has a reducing end, maltose is a reducing sugar.

The source of maltose in food products is incorporation of hydrolysis products of starch (Section 1.8.1), primarily glucose syrups. Glucose syrups can be made from any starch via extensive hydrolysis using enzymes, hot solutions of acids, or a combination of the two. They are composed of varying proportions of monosaccharide (D-glucose/dex-
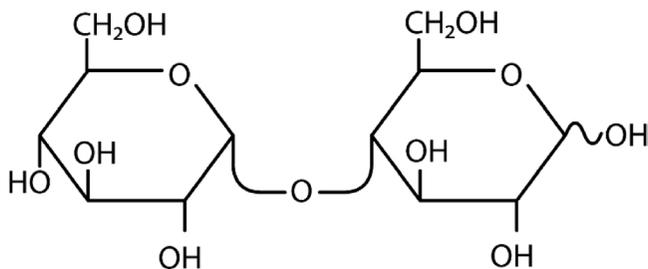
FIGURE 1.6. Maltose.

trose), disaccharide (maltose), and tri-, tetra-, penta-, hexa-, and higher oligosaccharides, all of which (other than dextrose) are known as maltooligosaccharides because they arise from starch (Section 1.8.1). High-maltose syrups that contain more than 50% maltose are available. Like lactose, maltose is used only sparingly. It is used as a mild sweetner. It is the starting compound for the production of malitol (Section 1.5.3.4).

1.4.1.4. Trehalose

Trehalose (Kappas, 2007; Richards and Dexter, 2012) is not yet used extensively, but is included here because of its claimed unique properties. The uniqueness claimed is that it stabilizes proteins, including enzymes (Heljo, Jouppila, Hatanpaa, and Juppo, 2001), and protects them from denaturation during both freeze-thawing and heating. It is also claimed to reduce retrogradation of starch (Section 1.4.3.1) and thereby to extend the shelf life of bakery products, to preserve cell structure during freezing, and to preserve flavors and color, especially in frozen products. Its sweetness is about 40% that of sucrose. It has a low degree of hygroscopicity, and glasses made from it have a relatively high glass transition temperature. It is non-reducing because it contains two glucopyranosyl units linked head-to-head, i.e., anomeric (hemiacetal) hydroxyl group-to-anomeric hydroxyl group. Because, like sucrose, it is a non-reducing disaccharide, it does not initiate Maillard browning reactions. The commercial form, made enzymically from starch, is α,α-trehalose (Figure 1.7), which contains two conjoined α-D-glucopyranosyl units.

1.4.2. Other Oligosaccharides

Oligo- and polysaccharides are characterized in part by their degrees
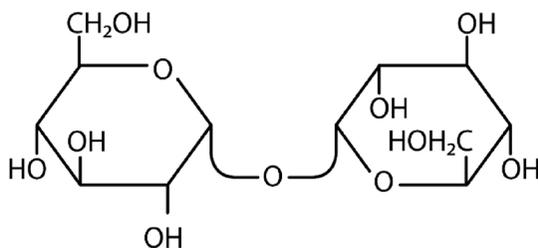
FIGURE 1.7. α,α-Trehalose.

of polymerization (DP). As already mentioned, the DP is the number of monosaccharide units in a molecule. Sucrose, lactose, and maltose, for example, all have a DP of 2. With the exception of a few di-, tri-, and tetrasaccharides, the DP of a product is an average number for a population of molecules. Those food ingredients containing oligosaccharides other than sucrose and lactose are primarily products of hydrolysis of polysaccharides (Section 1.8). Essentially all glucose syrups contain maltooligosaccharides, often as the predominate component (Section 1.8.1).

Other products of the hydrolysis of starch are the maltodextrins, which are mixtures of maltooligosaccharides (Section 1.8.1). One way of characterizing the hydrolysis products of starch is by determining their dextrose equivalency (DE), which is a measure of the product's reducing power as a percentage of the reducing power of dextrose (D-glucose). The starch polymer molecules, like all polysaccharides, have only one reducing end-unit and are so large that they have no measurable reducing power. However, hydrolytic cleavages create molecules with reducing ends—one new reducing end for each cleavage. Finally, the polymer molecules are completely converted into dextrose, which by definition has a DE value of 100. The DE values of syrups and maltodextrins are rough measures of the extent of hydrolysis. DE values are related to the degrees of polymerization via the equations $DE = 100 \div DP$ and $DP = 100 \div DE$, with the understanding that both values are average values for populations of molecules. Syrups have DE values of > 20 and may be as high as 95 or more. Maltodextrins by definition have DE values of < 20, with the majority of constituent maltooligosaccharides having DPs of from 7 to 20. Hydrolysis to DE 20-35 (average DP 5-3) produces mixtures of molecules that, when dried, are called glucose syrup solids or, in the United States, corn syrup solids.

Another source of oligosaccharides is a family of compounds known as fructooligosaccharides (FOS) (Section 1.8.2). FOS are produced by partial hydrolysis of the polysaccharide inulin and are used as prebiotics and sources of dietary fiber. A typical FOS will contain about 85% tri-, tetra-, and pentasaccharides (DP 3, 4, and 5).

Yet another source is a special group of compounds known as cyclodextrins, the commercial one being β-cyclodextrin (βCD) (Lincoln and Easton, 1998; Szejtli, 2004; Szente and Szejtli, 2004; Dodzluk, 2006; Hedges, 2009). βCD is a cyclic oligosaccharide composed of seven (1→4)-linked α-D-glucopyranosyl units. It is derived from starch, βCD and has the ability to complex hydrophobic molecules, such as those in essential oils and other flavor and aroma compounds, convert them

to stable powders, and protect them from oxidation, volatilization, and thermal and light-induced degradation. The protected molecules are released when the complexes are placed in an aqueous system.

1.4.3. Polysaccharides

Polysaccharides are polymeric carbohydrates composed of monosaccharide units that have DPs of from about 35 to probably at least 100,000 (BeMiller, 2007, pp. 93–117). Some, particularly the starches, are natural components of food ingredients such as flours. Others, generally classified as hydrocolloids or food gums, are added to provide specific functionalites (Misaki, 1993; Whistler and BeMiller, 1993). All, except for the starches (but including a portion of certain starches called resistant starch), are non-digestible and classified as dietary fiber. Polysaccharides are primarily used to impart viscosity and to produce thickening to aqueous systems and to control or modify the flow characteristics (rheology) of those systems (Morris, 1993); but they also provide a wide range of beneficial functionalities (Dickinson, 1993; Morris, 1993; Clark, 2000; Wang and Cui, 2005; BeMiller, 2007, pp. 119–171). They may be used to form gels (Clark, 2000) with specific characteristics, to hold moisture, to provide bulk and body, to stabilize emulsions (Dickinson, 2009), suspensions, foams, and proteins, to provide freeze-thaw and low-temperature stability (inhibit syneresis), to form films (coat), to act as binders, and for other reasons. While the various polysaccharides are presented below individually (for the most part), hydrocolloids in combination with starches, cellulose products, and other hydrocolloids are often employed in the preparation of food products because of the synergistic or additive effects realized with such combinations. The properties of all food systems containing polysaccharides are a function of other ingredients present—added, ideally, after the polysaccharide is dissolved.

1.4.3.1. Starches

On the general topic of starches see Whistler, BeMiller, and Paschall (1984); Eliasson (2004); Jane (2004); Liu (2005); Eliasson and Gudmundsson (2006); BeMiller (2007, pp. 173–223); BeMiller and Whistler (2009); Bertolini (2010).

There are many commercial sources of starches including those from cereals (such as corn/maize, rice, and wheat starches), roots and tubers

(such as potato and tapioca/cassava starches), legume seeds (such as mung bean and yellow pea starches), and others such as sago starch. Though each starch is unique in almost every measurable aspect, there are certain features common to all. All native starches are granular. Starch granules are cold-water insoluble and partially crystalline. They do not release their constituent polysaccharide molecules to provide thickening, texture, and body until they are heated above a temperature known as the pasting temperature, usually with some shear (Biliaderis, 2009; Colonna and Buleon, 2010). Preceding the pasting temperature is a temperature known as the gelatinization temperature—the temperature range at which granules lose their crystalline order.

A hot starch paste contains at least partially dissolved starch granules in a continuous phase, and granule fragments and perhaps swollen granules in a discontinuous phase, the proportions of each depending on the starch used, the cooking conditions, and the amount of water present—e.g., a dough vs. a system with excess water for pasting the starch. Of course, also present will be any other substances/ingredients in the aqueous system that was heated.

So-called normal starches have two polysaccharide molecules in their granules. One is an essentially linear molecule termed amylose. The structure of amylose is mostly that of α-D-glucopyranosyl units joined by (1→4) glycosidic linkages. Some amylose molecules in any preparation are slightly branched with the same type of branching found in amylopectin. The so-called waxy starches contain only amylopectin. High-amylose starches (or, in the case of corn, amylomaize starch) contain > 30% apparent amylose, i.e., amylose as measured by iodine binding; at least, some of the iodine bound by these starches is undoubtedly due to binding by long linear chains in branched molecules. Yet these molecules in amylomaize starches (50% and 70% amylose) impart to the starches the cooking, solubility, and retrogradation characteristics of linear molecules.

As a hot, viscous paste or baked dough cools, a process known as retrogradation takes place. Retrogradation is a polymer crystallization process. Initially, the amylose molecules form crystalline junction zones, which, when excess water is present, result in gel formation. With storage, the slower process of retrogradation of amylopectin molecules takes place. All-amylopectin starches, such as waxy maize starch, form weak gels. Retrogradation also occurs in baked products, contributing to staling (Gray and BeMiller, 2003).

In a food system, other ingredients, such as sugar, emulsifiers, and

salt, influence the processes of gelatinization, pasting, and retrograda- tion, either enhancing or retarding the specific process.

Starch pastes are generally strongly thixotropic, although the specific rheological attributes of pastes and gels depend on the specific starch used, its concentration, how the paste or gel was prepared (time, temperature, and amount of shear during cooking), time and conditions of storage, and the presence of other ingredients—again remembering that no two starch- es are alike in their behaviors and their paste/gel characteristics.

A somewhat more extensive, but not exhaustively detailed, descrip- tion of the processes of gelatinization, pasting, retrogradation, and gelation and the characteristics of starch pastes and gels is presented in BeMiller (2011). Most starches used as food ingredients have been modified chemically and/or physically to alter their cooking character- istics, their paste/gel behaviors, and/or their functionalities (Sections 1.6.1.3, 1.6.2.1, 1.6.3, 1.8.1).

1.4.3.2. Cellulose

Cellulose (BeMiller, 2007, pp. 225–243) is largely used as a food ingredient in the form of cellulose ethers (Section 1.6.2.2). Powdered cellulose itself may be incorporated into a food (particularly a bread) to increase its dietary fiber content and/or to reduce its caloric value, to hold moisture, to increase cling in sauces and dressings, or as an anti- caking agent.

Treating cellulose with an acid so that amorphous regions are re- moved produces a family of products known as microcrystalline cel- lulose (MCC) (Buliga, Ayling, Krawczyk, and McGinley, 1998; Iijima and Takeo, 2000; Krawczyk, Venables, and Tuason, 2009; Tuason, Krawczyk, and Buliga, 2010). One group of microcrystalline celluloses known as powdered MCC contain particles < 0.1 μm in length. Pow- dered MCCs are used as anticaking agents and flavor carriers for grated and shredded cheese. They are also used as extrusion aids for expanded snacks and to make high-fiber and reduced-calorie bakery products.

A second type of MCC, called colloidal MCC, requires higher shear to tear apart weakened cellulose microfibrils, producing colloidal-sized (< 0.2 μm) aggregates. Neither powdered cellulose nor powdered or colloidal MCC are water soluble. However, when dispersed in an aque- ous system with high shear, colloidal MCC has some of the attributes of water-soluble hydrocolloids. The dispersed microcrystals swell, result- ing in time-dependent viscosity development. Colloidal MCCs gener-

ally form thixotropic, heat-stable gels. They are also used to stabilize emulsions and foams, to prevent separation and whey off and to provide freeze-thaw stability and improve heat shock in ice creams and other frozen desserts, to extend or replace fats and oils (by mimicking the particle size of fat and oil droplets), to provide a creamy mouthfeel and body in dairy creams, to enhance cling in batters, to prevent boilout in bakery fillings, and as a carrier for essential oils and other flavors.

1.4.3.3. Hydrocolloids

The hydrocolloids, also known as food gums (Whistler and BeMiller, 1993; Stephen, 1995; Dumitriu, 1998; Phillips and Williams, 2009; Hoefler, 2004; Doublier and Cuvelier, 2006; Williams and Phillips, 2009a; Imeson, 2010), are a structurally diverse group of non-starch polysaccharides. Some protein ingredients are also classified as hydrocolloids (Phillips and Williams, 2009). The only thing the various hydrocolloids have in common is that they are water soluble and are approved for use as food ingredients. All, except gum arabic, are basically linear. Although many have short (monosaccharide, oligosaccharide, or other) groups attached to a linear backbone and are technically branched polymers, they behave as linear polymers. Some are available in different viscosity grades. With the exception of the low-viscosity grades of some hydrocolloids and gum arabic, all will thicken aqueous systems at low concentrations (usually used at levels of < 2%) with shear-thinning rheology (Morris, ER, 1993, 1995; Lapasin and Pricl, 1995; BeMiller, 2007, pp. 119–171). Among them are modified celluloses (cellulose ethers), which are described in Section 1.6.2.2. Others are briefly reviewed here.

Anionic polysaccharides have the ability to form protein-polysaccharide complexes and coacervates which have different solubility, rheological, interfacial—i.e., emulsifying (Dickinson, 2009)—and foam formation properties than do the hydrocolloids themselves, and may provide encapsulation capability to the hydrocolloid and texturization of the food product (Schmitt, Aberkane, and Sanchez, 2009). Many hydrocolloids can provide emulsion stability, although not always by the same mechanism and not as effectively as proteins, except for xanthan (Dickinson, 2009).

Guar and locust bean gums. The polysaccharides of guar and locust bean (also called carob) gums belong to a family of polymers called galactomannans (Maier, Anderson, Karl, Magnuson, and Whistler, 1993;

Reid and Edwards, 1995; BeMiller, 2007, pp. 245–254; Cui, Ikeda, and Eskin, 2007; Wielinga, 2009, 2010). Commercial products are not purified polysaccharides, but rather the ground endosperms of guar or locust bean seeds. A formal name ending in –an indicates that the compound is a polysaccharide, so a galactomannan is a polysaccharide composed of galactosyl and mannosyl units (BeMiller, 2001). Galactomannans have a main chain of (1→4)-linked β-D-mannopyranosyl units. Some of the main-chain units have single α-D-galactopyranosyl units attached to their O-6 atom. Guar gum molecules have galactosyl side units on about 56% of the main chain units. Locust bean gum has side units on only about 25% of the main chain units, and the branches occur in clusters along the main chain. The branching of tara gum, a minor hydrocolloid is intermediate between that of guar and locust bean gums. Guar gum, widely used as a thickener, is available in low-, medium-, high-, and very high-viscosity types. The rheology of its solutions is pseudoplastic rheology (Morris, E.R., 1993, 1995; Lapasin and Pricl, 1995; BeMiller, 2007, pp. 137–145). It is also used to bind water, i.e., to reduce water migration.

Because the polysaccharide molecules of locust bean gum (LBG) have their galactosyl side units in clusters, their backbone mannan chains have unsubstituted (or "naked") regions that can associate with each other. As a result, LBG is only slightly soluble in room-temperature water. Heating an aqueous dispersion to about 85°C is required for good dissolution. Its long unsubstituted regions can interact with certain other hydrocolloid molecules to form the junction zones of a three-dimensional network and bring about gelation (Williams and Phillips, 1995; Walter, 1998). In combination with κ-carrageenan (see under Section 1.4.3.3 Carrageenan), gels are formed that are stronger, less brittle, and more elastic than gels made with κ-carrageenan alone. The LBG-κ-carrageenan combination produces retort-stable, chewy gels, so the combination is frequently used in pet foods. LBG also interacts with xanthan (see under Section 1.4.3.3 Xanthan) in this way, but neither xanthan nor LBG by themselves form gels, so comparisons cannot be made. About 85% of LBG is used in dairy and frozen food products, most often in combination with κ-carrageenan (dairy products) or xanthan, but at times with CMC.

A hydrocolloid product somewhat like LBG is made by treating guar gum with an α-galactosidase to remove some of the galactosyl side units, but such a synthetic product is not as effective in its synergism with κ-carrageenan as is LBG itself.

Xanthan. Xanthan (known commercially as xanthan gum) is a microbial polysaccharide with a particular helical structure in which trisaccharide side-chain units interact with the backbone chain in a way that makes the molecules quite stiff (Challen, 1993; Kang and Pettitt, 1993; Morris, VJ, 1995; Nussinovitch, 1997; Stokke, Christensen, and Smidsrød, 1998; BeMiller, 2007, pp. 263–270; Sworn, 2009a, 2010). As a result, xanthan solutions are quite viscous and very pseudoplastic, and the viscosities of its solutions are unusually stable to heat and acidic conditions. The high degree of pseudoplasticity of its solutions means that they are not perceived in the mouth as being slimy (Morris, E.R., 1995). Its solutions also have a relatively high yield value, which is the force that must be applied before the solution begins to flow. This latter property makes it essentially ideal for stabilization of emulsions and suspensions. To stabilize an oil-in-water emulsion, the resistance of movement of the oil droplets must prevent them from rising to the surface. Likewise, to stabilize a suspension, the resistance to downward movement must be greater than the downward force of gravity. The high yield values of xanthan solutions provides these functionalities. As a result, xanthan is employed in such products as pourable salad dressings. The fact that the viscosities of solutions of xanthan do not change with temperature changes makes it particularly useful in the same salad dressings, chocolate syrups, and like products that need to pour as easily when taken from the refrigerator as they do at room temperature. It is also used, often in starch-based formulations, in sauces that need cling and/or freeze-thaw stability and in bakery mixes. It is used in combination with locust bean gum in frozen novelties.

Carrageenans. A carrageenan is a member of a family of polysaccharides extracted from certain species of red seaweeds/algae (Therkelsen, 1993; Piculell, 1995; Nussinovitch, 1997; Thomas, 1997; BeMiller, 2007, pp. 279–291; Campo, Kawano, de Silva, and Carvalho, 2009; Imeson, 2009; Blakemore and Harpell, 2010). They are almost always described in terms of three idealized types: kappa-, iota-, and lambda-carrageenan. All three types are sulfated, linear galactans. Therefore, all three types are anionic. The contents of half-ester sulfate groups ranges from 18 to 40 wt% and are in the order κ-type > ί-type > λ-type. The κ- and ί-types contain galactosyl units with a C3 to C6 ether bond (3,6-anhydro ring) in approximately every other chain unit. The half-ester sulfate groups make the molecules more hydrophilic and the 3,6 ether groups make the molecules less hydrophilic, so the solubilities of the three ideal carrageenans is the order κ-type > ί-type > λ-type,

which is also the order of decreasing sulfate group content, and the gelling ability and gel strength is in the order í-type > κ-type, which is also the order of decreasing 3,6-anhydro ring structure content. λ-Type carrageenan is non-gelling. In general, 3,6-anhydrogalactosyl units are required for gelation, while solubility, gel texture, and protein reactivity are related to the degree of sulfation.

Gels with a wide range of characteristics can be made with κ- and í-type carrageenans plus various cations (K^+, Ca^{2+}, proteins) by varying the type and concentration of the hydrocolloid preparation, the type and concentration of the cation, and the method of preparation and by adding other ingredients, such as sugar. Kappa types form the strongest gels with potassium ions; these gels are firm and brittle. Iota types form the strongest gels with calcium ions; these gels are soft and elastic and are rather freeze-thaw stable. Both types exhibit a high degree of synergism with locust bean gum (Williams and Phillips, 1995; Walter, 1998).

Carrageenans are often used because of their ability to form gels with both milk and water that do not require refrigeration because they do not melt at room temperature, but neither do they melt in the mouth. κ-Type carrageenans interact with milk proteins; particularly do they associate with the κ-casein micelles of milk, forming weak, thixotropic, nonmelting, pourable gels.

Many different commercial carrageenan products are made by blending either the seaweeds from which the carrageenans are extracted or the extracts themselves to produce products with different properties and/or by adding salts—especially potassium and calcium salts—and sugar to standardize gel or viscosity characteristics for a specific application. As a result, a large number of carrageenan products find use in a variety of applications. A carrageenan product is universally used as an ingredient in any dairy product, including dry mixes. For example, a carrageenan product is commonly used in ice creams, often in combination with LBG, to prevent separation and whey off. Carrageenan products are also commonly used as thickeners, stabilizers, and syneresis inhibitors in other dairy products. Carrageenan products increase the sensory perception of creaminess in low-fat dairy products. Processed meat products often contain a carrageenan product to improve and control textural properties, especially in low-fat products where they hold moisture, resulting in purge and cooking loss reduction, and maintain softness. A carrageenan product is also frequently used in the brines used in the preparation of sliceable meat products to hold the brine and improve sliceability, preferably without a large increase in brine viscosity.

Also available is a carrageenan product which is not an extracted polysaccharide, but rather a κ-type, ί-type, or a combination of both in a flour of the seaweed itself minus the low-molecular-weight components that were soluble in the potassium hydroxide solution used to make the product (Bixler and Johndro, 2000).

Alginates/Algins. Alginates are linear, anionic polysaccharides extracted from brown seaweeds (Sanderson and Ortega, 1993; Moe, Draget, Skjå-Braek, and Smisrød, 1995; Nussinovitch, 1997; BeMiller, 2007, pp. 293–301; Draget, 2009; Helgerud, Gåserød, Fjaereide, Andersen, and Larsen, 2010). They are anionic because they are composed exclusively of two uronic acid units, both in a pyranosyl ring form. Uronic acids are monosaccharides which contain a carboxyl group rather than a primary hydroxyl group. The two uronic acid units in alginates are D-mannuronic acid units in the β anomeric (βMan*p*A) configuration and L-guluronic acid units in the α anomeric configuration (αLGul*p*A). The ratio of the two units depends on the source, i.e., species, of brown algae and the conditions under which a particular species grew, and can vary greatly. The ratio of βMan*p*A units to αLGul*p*A units can vary from < 0.5 to > 2.0 in commercial preparations. In every case, however, the two units are arranged in blocks, with blocks of only βMan*p*A units, blocks of only αLGul*p*A units, and blocks containing both units. Alginates with high contents of αLGul*p*A unit blocks produce gels of high strength with calcium ions. Blending of alginates from different sources is done to create preparations with desired properties and functionalities. The most common salt form is the sodium salt form. Potassium and ammonium alginates are also available. All three forms are water soluble. Addition of small amounts of calcium ions to solutions of these alginates effects an increase in viscosity. Slightly higher concentrations of Ca^{2+} ions produce thixotropic solutions; even higher concentrations convert alginate solutions into permanent gels.

Alginates are also available as propylene glycol esters, called propylene glycol alginates (PGA). PGAs can vary in the degree of esterification (from 50 to 90% of the carboxyl groups esterified) and molecular weight. PGAs with > 60% esterification form solutions that simply thicken upon addition of Ca^{2+}, while those with < 60% esterification form solutions that become thixotropic upon addition of Ca^{2+}. PGAs have emulsifying and emulsion-stabilizing properties because of their surface activity and high molecular weight. They are very commonly used in combination with xanthan in the preparation of pourable salad dressings.

The primary use of sodium alginates is to form gels upon addition of Ca^{2+} ions. There are three ways to produce calcium alginate gels. First, in diffusion setting, a mixture containing sodium alginate is immersed in some way in a solution of calcium ions, such as a calcium chloride solution. When the calcium ions come into contact with the surface of the mixture, a skin is formed. Then, more Ca^{2+} ions diffuse from the outside in. This process may be terminated when the particle or drop still has a liquid center. Second, internal setting involves slow release of calcium ions within a mixture containing an insoluble calcium salt, a sequestrant, and an acid to release the calcium ions. Third, setting by cooling involves cooling a mixture of dicalcium phosphate, calcium lactate, calcium citrate or calcium gluconate, a slightly soluble acid such as adipic acid or GDL, and a sequestrant in hot water. In all three cases, the gels that are formed are heat stable and can be used, for example, as gelled centers in products to be baked. Alginates also interact with positively charged protein molecules. Alginate-whey protein, minced fish, and comminuted meat complexes are formed at pH values of ca. 5. Soft, thixtropic, nonmelting gels are formed when the pH of an alginate solution is lowered sufficiently to convert a sufficient portion of the carboxylate ($-COO^-$) groups to the less polar carboxylic acid ($-COOH$) groups. Propylene glycol alginates are stable in acidic systems, less reactive with calcium ions, and have interfacial properties that make them suitable for stabilizing emulsions.

Pectins. Like alginates, pectins are mostly used because of their ability to gel aqueous systems. Pectins (May, 1990; Rolin, 1993; Voragen, Pilnik, Thibault, Axelos, and Renard, 1995; Nussinovitch, 1997; Rolin, Nielsen, and Glahn, 1998; MacDougall and Ring, 2004; BeMiller, 2007, pp. 303–311; Endress and Christensen, 2009; Brejnholt, 2010) are polymers of D-galacturonic acid with the galacturonic acid units being in the α-D-pyranosyl ring form. They are extracted from citrus peel or apple pomace.

In all pectins, some of the D-galacturonic units occur as their methyl esters. Those pectins in which more than half of the carboxyl groups are methyl esterified are termed high-methoxyl (HM) pectins, and those with less than half of the carboxyl groups being present as methyl esters are known as low-methoxyl (LM) pectins. Amidated pectins are low-methoxyl pectins in which up to 25% of the carboxyl groups are present as amides ($-CONH_2$). The remainder of carboxyl groups in all three categories of pectins are either present as sodium carboxylate or carboxylic acid groups.

Pectins are primarily used to make spreadable gels, i.e., jams, jellies, marmalades, and preserves. The standard types of these products are made with a HM pectin product, about 65% sugar (required), and a pH of 2.5–3.8 (required). LM pectin solutions, like alginate solutions, form gels upon addition of calcium ions and are used in this way to form jams, jellies, and marmalades without added sugar. Amidated pectins are more sensitive to Ca^{2+} than are LM pectins. Gels made from any of the three types of pectin are thermoreversible, unlike calcium alginate gels. Pectins are well suited for acidic foods because of their stability to low pH values and are also used in acidified beverages (including LM pectin in milk products with pH values below 4.0–4.5) and confections.

Gellans. Gellans, known commercially as gellan gums (Kang and Pettit, 1993; Moritaka, Nishinari, Nakahama, and Fukuba, 1993; Sanderson and Ortega, 1993; Vendrusculo, Pereira, and Scamparini, 1993; Morris, V.J., 1995; Nussinovitch, 1997; BeMiller, 2007, pp. 271–274; Sworn, 2009b; Valli and Clark, 2010), are used for their gel-forming ability. Like xanthan, gellans are extracellular, anionic, microbial polysaccharides. They have a negative charge because every fourth glycosyl unit is an uronic acid unit. Native gellan (known commercially as high-acyl gellan) contains two kinds of ester groups. High-acyl gellan is used at low concentrations (0.02–0.1%) as a suspension-stabilizing, thickening agent. Some of the gellan offered for food use has been de-esterified. Removal of the ester groups dramatically changes gel properties. The de-esterfied form, i.e., low-acyl gellan, can gel aqueous systems at concentrations of 0.15–1.0% in the presence of either monovalent or divalent cations, with Ca^{2+} ions being about 10 times more effective than Na^+ ions. Shearing during cooling of a hot gellan solution prevents the normal gelation mechanism from occurring and produces a smooth, homogeneous, thixotropic fluid that stabilizes emulsions and suspensions very effectively. Gentle agitation of a weak gellan gel will also disrupt the gel structure and turn the gel into a smooth, pourable, thixotropic fluid with excellent emulsion and suspension-stabilizing properties.

Gum arabic. Gum arabic (Acacia gum) (Glicksman, 1983; Nussinovitch, 1997, 2010; BeMiller, 2007, pp. 313–319; Williams and Phillips, 2009b; Thevenet, 2010) is unique among the hydrocolloids, with one exception, the exception being certain octenylsuccinylated starch products (Section 1.6.1.3). Corn fiber gum (see under Section 1.4.3.3 Arabinoxylans and beta glucans) and modifications of it have the potential of being a substitute for gum arabic.) One uniqueness of gum arabic is that it can be dissolved in water at concentrations of $> 40\%$ without produc-

ing high viscosity. In general, concentrations 20–40 times greater than those of other hydrocolloids are required to produce equivalent viscosities. A second uniqueness is that it is an excellent emulsifying agent for citrus and other flavor oils. In this application, it both reduces the interfacial tension and forms thick, sterically stabilized layers around the oil droplets. Because of this property, gum arabic can stabilize not only concentrated (10–20% by weight) flavor oil emulsions, but also the highly diluted emulsions in beverages. Gum arabic is also relatively nonhygroscopic, heat stable, and produces solutions of low viscosity with Newtonian rheology. Because of these properties, flavor oil emulsions prepared with gum arabic can be spray-dried to produce an encapsulated flavor product, one of its main uses. Another attribute is its ability to dissolve quickly in water and release the encapsulated oil. Yet another uniqueness is its compatibility with high concentrations of sugar. Its primary use is in confections such as caramels, toffees, jujubees, and pastilles, where it prevents sugar crystallization and prevents fat migration.

Inulin. Inulin preparations (Praznik, Cieślik, and Huber, 2003; Franck and Bosscher, 2009; Meyer and Blaauwhoed, 2009; Wouters, 2010) are mixtures of small polysaccharide molecules composed of β-D-fructofuranosyl units. Some of the inulin molecules and even smaller molecules related to them that are classified as fructooligosaccharides are terminated at their reducing end with a sucrose unit. Inulin is used as a functional food ingredient (Roberfroid, 2004; Madrigal and Sangronis, 2007; Kalyani Nair, Kharb, and Thompkinson, 2010), i.e., as a source of soluble dietary fiber, and is frequently converted into oligosaccharides (Section 1.8.2).

Arabinoxylans and beta-glucans. Up until now, arabinoxylans have been naturally present food ingredients rather than added ingredients (Izydorczyk and Biliaderis, 1995, 2000, 2007; Izydorczyk, 2009; Autio, 2006). They are a group of hemicelluloses that have a linear xylan backbone chain to which are attached mono- or disaccharide branches. The branch units may contain several different sugars but are predominately L-arabinosyl units, hence the classification as arabinoxylans. The arabinoxylans of most interest in food technology are those of the cereal grains, especially the wheat flour arabinoxylans, which are also classified as nonstarch polysaccharides (NSP) and as pentosans.

Wheat flour arabinoxylans/pentosans are divided into two classes: water-soluble and water-insoluble. Both classes have been studied with respect to their structures and their influence on breadmaking and bread

quality. Arabinoxylans are the major NSP of wheat endosperm and flour. They constitute 60–70% of wheat endosperm cell walls (and bran), but less than 2% of wheat flour (water-soluble types being ~0.7%, water-insoluble types being ~1.3%, although reported percentages vary). Arabinoxylans make up ~8% of rye flour.

Both soluble and insoluble arabinoxylans effect the preparation, quality, and shelf-life of bakery products. They are important primarily because of their great propensity to sorb and bind water, even though they are present in only small amounts. The high water-holding capacity of cross-linked arabinoxylans (via oxidative coupling of the ferulic acid ester groups present in their structures) affects distribution of moisture among bread dough constituents, the rheological properties of the dough, gas rentention/loaf volume, and the texture and rheological properties of the baked product because it has a negative impact on the formation of the gluten network.

Certain enzyme preparations may be used as flour improvers. Xylanases or hemicellulases added to flours break down the pentosans and decrease their water-sorbing and binding capacities, improve bread-making quality, and reduce staling (Rouau, 1993; Courtin and Delcour, 2002; Jiang, Li, Yang, Li, and Tan, 2004). The end result of treatment with a xylanase preparation is release of water for gluten formation. An important use of such enzymes is to delay syruping during storage of refrigerated doughs (Courtin, Gys, and Delcour, 2006).

Another polysaccharide naturally present in cereal grains is β-glucan (Izydorczyk and Biliaderis, 2000; Autio, 2006; Lazaridou, Biliaderis, and Izydorczyk, 2007; Izydorczyk and Dexter, 2008; Stevenson and Inglett, 2009). Oat and barley flours, and sometimes extracts from them, are used as ingredients because cereal β-glucans are quite as effective as soluble dietary fiber.

One hemicellulose that appears to have promise as a food gum, especially as a replacement for gum arabic, is corn fiber gum. The seed coats/brans off all cereal grains are rich sources of pentosans/hemicelluloses. Isolated from the "corn fiber" fraction in the wet-milling process used to prepare corn/maize starch, an arabinoxylan called corn fiber gum is, like gum arabic, a protein-polysaccharide with the protein part being hydrophobic (Yadav, Nunez, and Hicks, 2011). When isolated in the proper way, it has emulsifying properties as good as or better than those of gum arabic (Yadav, Moreau, Hotchkiss, and Hicks, 2012). It has also been investigated for its effect on expansion of extruded snacks and as a source of soluble dietary fiber.

1.5. REACTIONS OF CARBONYL GROUPS

1.5.1. Nonenzymic Browning

It is important to food processors that the aldehydo group of aldoses reacts with primary and secondary amino groups of amino acids, peptides, and proteins and, thereby, initiates the reaction variously known as the Maillard reaction and nonenzymic or nonenzymatic browning (Davidek and Davidek, 2003; Nursten, 2005; BeMiller, 2007, pp. 47–57). The first reaction intermediate is a Schiff base, but the reaction continues through many intermediates which polymerize to form a variety of brown or black compounds collectively called melandoins and a variety of compounds that impart flavors and aromas. Because the Maillard reaction sometimes produces unwanted color and/or flavor, use of reducing mono- and oligosaccharides in food systems containing proteins may be undesirable. On the other hand, this type of browning is desired in products that are baked, fried, or roasted and in chocolates, caramels, toffee, fudges, soy sauce, and beer. By far, the monosaccharide most likely to be present in a food product is D-glucose/dextrose.

1.5.2. Oxidation of the Aldehydo Group (Glucono-Delta-Lactone)

The aldehydo group of aldoses is quite easily oxidized (as are the reducing ends of oligo- and polysaccharides, though such is seldom practiced). The most extensively practiced commercial oxidation of a reducing sugar is that of D-glucose. In that case, it is not the aldehyde group that is oxidized, but rather its hemiacetal (pyranose ring) form (BeMiller, 2007, pp. 28–29). The product of this oxidation is the cyclic ester D-glucono-1,5-lactone (glucono-delta-lactone, GDL) (Figure 1.8). An enzyme, glucose oxidase, is used for the commercial production of GDL. GDL is used where a slow production of acid is required, such as in chemical leavening agents, and in the preparation of some dairy products, since spontaneous ring-opening in water forms D-gluconic acid, and in certain fish and meat products (as a preservative).

1.5.3. Reduction of Carbonyl Groups

Reduction, i.e., hydrogenation, of the aldehydo (usually) or keto group of aldoses or ketoses, produces a hydroxyl group. Members of the class compounds produced in this way have hydroxyl groups on

Carbohydrates as Flavor Delivery Systems

GARY A. REINECCIUS, Ph.D.

4.1. INTRODUCTION

THE food and flavor industries are constantly looking for better delivery systems to improve flavoring quality and enhance the sensory experience of the consumer. But flavors are inherently difficult to deliver in food applications, because many flavor components are susceptible to oxidation, evaporation or chemical reaction. Food flavorings can take several different forms: liquids, emulsions, or dry forms. The liquid forms are often compounds dissolved in either a water-soluble (such as ethanol or propylene glycol) or an oil-soluble solvent (vegetable oil, triethyl citrate or triacetin). There is, really, no delivery technology involved in these flavorings other than choosing the flavor solvent to be compatible with the food application; for example, an aqueous-based food would use an aqueous-soluble flavor solvent.

Most flavoring materials have limited or, effectively, no solubility in water. Putting these materials in an emulsion form permits us to use an inexpensive solvent for delivery—water. This certainly has some application in terms of adapting flavoring materials to a given food application: for example, putting water insoluble flavoring materials into a mouthwash to make it clear, or into a beverage where we want the

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system to be cloudy in order to give the appearance of juice solids being present. There are some studies in the literature which suggest that delivering flavors in an emulsion form can offer greater stability to the flavor due to oxidation, or to other types of deterioration, as opposed to simply using it in a pure liquid form. There are also claims of controlling the delivery of flavorings though the use of emulsion forms. However, when one talks about controlled release flavor delivery systems, one most commonly thinks of encapsulation. All encapsulation processes require that an emulsion be formed prior to or during the encapsulation process. Thus there is great interest in emulsion formation in flavor delivery.

In this chapter we will primarily consider how carbohydrates serve in flavor delivery systems. While a limited number of other food ingredients serve this purpose, no other class of food ingredients serve it so well, or as broadly, as carbohydrates. The carbohydrates used are chosen based on compatibility with the process, cost, flavor type being encapsulated, stability factors (the flavorings may be prone to oxidation or loss through diffusion), legal constraints, religious constraints, allergenicity, and functionality in the final application. For example, if one wishes to have the flavoring used in an all-natural food, a natural wall material such as gum acacia will be needed. If the flavoring is water soluble, one can use a non-emulsifying wall material, such as a maltodextrin, in encapsulation processes. However, if the flavoring is insoluble, an emulsion must be made, thereby dictating the use of an emulsifying food polymer such as modified starch, gum acacia or a protein. If an encapsulated flavoring is to be used in a kosher or Halal product, then gelatin is not acceptable. Generally, numerous considerations must be taken into account in this selection: some will be discussed in the following sections. The reader is encouraged to consult one or more of the following reviews in this area for greater detail—Wandrey *et al.* (2009), Nedovica *et al.*, (2011), Sri *et al.*, (2012), Nussinovich (2003) or Ubbink and Schoonman (2005).

4.2. PARAMETERS AFFECTING FLAVOR DELIVERY

4.2.1. Emulsification

The importance of the emulsifying properties of flavor carriers in flavor delivery depends upon the type of flavoring delivered, the delivery process, and the final application. As noted earlier, there is no need to

emulsify a water-soluble flavoring; but a note of warning must be given here. A flavoring labeled as "water-soluble" is generally formulated in either alcohol or propylene glycol, and is readily dispersed in an aqueous food system at 0.1% or slightly higher usage levels. This does not mean that all of the flavor components in this flavoring are still soluble once they are placed in an aqueous system. This may mean placing the flavoring materials in an emulsion delivery system or an emulsion for a subsequent encapsulation process, for instance blending into an aqueous dispersion of gum acacia, at a 20% loading. One must be conscious of whether all of the components of a flavoring are truly soluble under manufacturing conditions and concentrations. Only then can one use a delivery system that has no emulsification properties.

If the flavoring, or any part of it, is insoluble in the system used for delivery, an emulsifying matrix is required. The food components typically considered as emulsifiers are proteins, mono/diglycerides, lecitins, chemically modified starches, or gum acacias. In traditional usage in flavor delivery, this list narrows to chemically modified starches and gum acacias. Proteins are too reactive with flavoring materials to be considered for general use (Charve and Reineccius, 2009; Ubbink and Schoonman, 2005) and the other smaller molecular weight emulsifiers are less effective for flavoring materials. Other plant materials, to be discussed later, often are discussed in research publications but have not found significant use in flavor delivery.

All encapsulation flavor delivery systems, other than cyclodextrin inclusion complexes, involve an emulsification process. This is because the manufacturing vehicle is water and the flavoring materials are typically insoluble in it. There are two primary capsule structures used in the industry: core and shell, and matrix encapsulation (Figure 4.1). Emulsification is required to distribute the insoluble flavoring material throughout the slurry of wall or encapsulation matrix (aqueous system—spray drying or extrusion processes), or to form the core mate-

FIGURE 4.1. *Distribution of flavoring in an encapsulated system. Left—matrix structure (white are flavor particles, shaded is wall material), Right—core and shell (white is flavor droplet, shaded is wall material).*

rial for the deposition of a wall material (coacervation process). More will be said about these processes later in the chapter. Emulsification is always needed for manufacturing purposes and in some cases upon final application: those that require a stable emulsion on reconstitution. In terms of final application, products such as dry savory mixes (gravies or sauces), baked goods (cake mixes or frostings), or confectionery products (candies), do not require the encapsulating material to provide significant emulsification properties in end use. However, a substantial portion of dry flavorings are used in dry beverage mixes. Such mixes must provide a one week shelf-life (no visible ring of separated flavor) following reconstitution. The encapsulating material used for this application must provide good emulsification properties. Because emulsification needs in processing may be very different than those required of the finished product, both must be considered.

If we go beyond the minimal emulsion considerations required in manufacturing, as well as the final application, other factors remain to be considered. A very important related issue is how emulsion quality influences our ability to retain flavorings during the dehydration process that is required in most encapsulation processes—for instance, in spray drying and extrusion processes. There is ample data in the literature showing that the retention of water insoluble flavorings are substantially improved if a good quality emulsion is prepared and used during the encapsulation process (Risch and Reineccius, 1986). The work of Soottitantawat *et al.* (2003) illustrates the benefit of a good quality emulsion to the retention of water insoluble volatiles, such as limonene, during spray drying. Good quality emulsions are those with a mean particle size of ca. 1 μm. These emulsions are prepared by using an emulsifying wall material, such as gum acacia, or a modified starch. One notes that retention is not necessarily improved by small mean particle sizes in more water soluble volatiles (e.g., ethyl propionate, Soottitantawat *et al.*, 2003).

As noted earlier, maltodextrins and corn syrup solids impart no emulsion stability other than that which is due to their high viscosity when initially manufactured. When an encapsulated flavor is based on maltodextrin or corn syrup solids, it is typically problematic in manufacturing and cannot be used for some applications (e.g., dry beverages) unless a secondary emulsifier is incorporated. Secondary emulsifiers are not commonly used with spray dried flavors; although an emulsifying agent, such as a modified starch or gum acacia, may be used in combination with maltodextrin or corn syrup solid flavor carrier. But they are

essential for the manufacture and stability of extruded flavorings. Both gum acacia and the modified food starches are excellent emulsifiers. The relative ability of corn syrup solids, traditional gum acacia, and a modified food starch to stabilize an orange oil emulsion prepared for spray drying is shown in Figure 4.2. This figure gives an initial turbidity (cloudiness) for the emulsion (time = 0), which indicates particle size of the flavor droplets and resultant cloudiness: higher turbidity indicates better emulsion quality. Turbidity during centrifugation indicates longer term stability.

In Figure 4.2 we see that modified food starch formed an emulsion that was substantially more turbid than either gum acacia or corn syrup emulsions (0.8 versus 0.6 or 0.3 absorbance units, respectively). Upon centrifugation (500 × g), all the emulsions cleared to some extent, due to separation. However, the modified food starch remained more stable than the gum acacia or corn syrup emulsions. These data point to a strength in using modified food starches for flavor encapsulation: their ability to form a stable flavor emulsion.

4.2.2. Particle Morphology

The physical and chemical attributes of the encapsulating material are all important in delivery systems. While processing technique es-
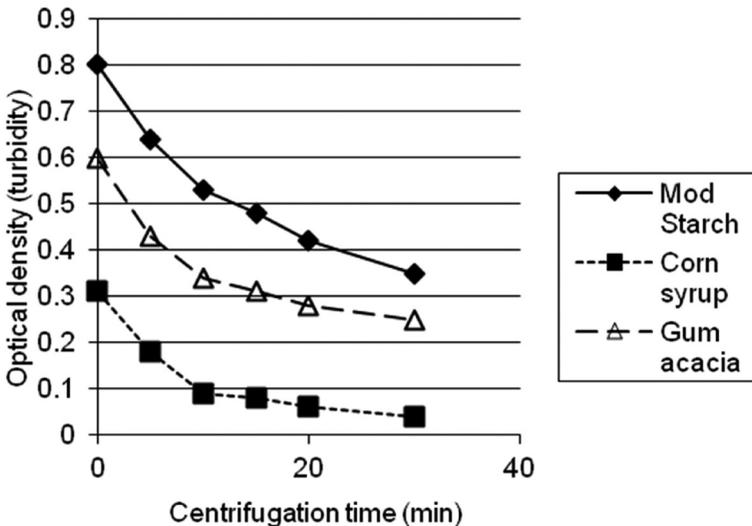
FIGURE 4.2. *Emulsion stability of various encapsulation materials (unpublished data, Reineccius).*

tablishes the gross particle structure, the encapsulation matrix largely determines performance attributes. Since most processes involve dehydration at some stage, the primary wall material must readily form a "quality" film and not be tacky when hot (stick to the walls during drying). It must retain flavors during and after encapsulation, protect the flavor during storage from reactions, and then release the flavor to the final food product on consumption. The particle structure will influence these performance criteria. The structure shown in Figure 4.4 is very undesirable. The combination of process and matrix composition has left unprotected oil (flavoring) on the surface of the particle, and cracks, or fissures, in the walls. The cracks and fissures allow oxygen to gain access to the interior of the particle, thereby reducing the diffusion path length for oxygen to interact with the flavoring, or for the flavoring to diffuse to a pathway to the particle surface, resulting to flavor loss. Entrapped air (closed porosity) is very common in spray dried particles, and this will enhance the diffusion of oxygen into, or flavoring materials out of the particle. There is evidence that closed porosity exceeding 20–30% of particle volume significantly enhances diffusion rates (Schoonman *et al.*, 2002).
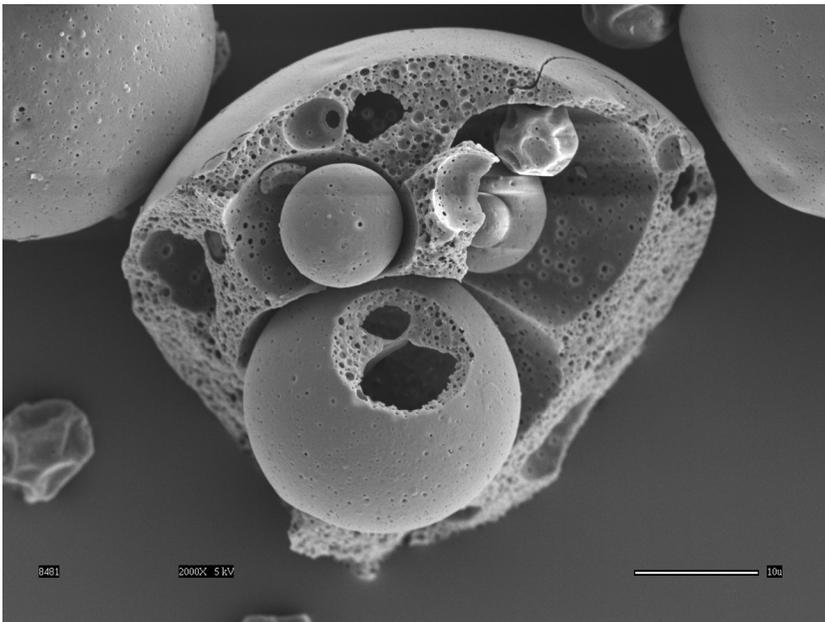
FIGURE 4.3. Undesirable particle structure.

4.2.3. Carrier Viscosity

The viscosity of a flavor carrier is critical to product performance. In fact, this is probably the most important single attribute that determines which materials can be used in encapsulation, especially by spray drying. The requirement is that materials used in encapsulation must have a low viscosity at high solids content. This rules out most of the hydrocolloids used in the food industry, effectively limiting us to gum acacia, mesquite gum, modified starches, starch hydrolysates and some simple sugars.

Viscosity exerts its influence by determining the infeed solids that one may work with in processing. For several reasons, one chooses to work with the highest infeed solids that one can pump and effectively atomize. Three of the most important are: the high production rates that result from high solids, because one is not evaporating a lot of water; the maximum flavor retention during drying; and lastly, the greater protection against flavor oxidation during storage (Reineccius, 2004; Charve and Reineccius, 2009). There is no logical option for not operating at the upper limits of solids content.

4.2.4. Retaining Flavors

The ability of an encapsulating material to capture or hold onto flavor compounds during the drying process and later during storage, is critical. If flavors are lost during drying, the resultant dry flavoring will be weaker, and potentially become imbalanced in character. The lighter, more volatile constituents will be preferentially lost during the process, with the resulting dry flavor lacking in the very light, fresh notes. A secondary concern centering on retention is that a flavor compound that is not retained in the powder is lost to the processing environment. In spray drying process, lost flavorings enter the dryer exit air which then must be removed by a costly scrubbing process in order to protect the environment. In extrusion, lost volatiles enter the workplace environment or the cold solvent used to solidify the particles. Therefore, encapsulation materials that offer poor retention during processing result in increased processing costs and decreased product quality. The issue of retaining flavor after drying must also be considered. As will be discussed later in this section, once encapsulated and in storage, some flavor molecules will be lost due to chemical reaction or diffusion through the encapsulation matrix and into the environment.

4.2.4.1. Retention During Manufacturing

Of the common edible films used in flavor encapsulation, modified food starches excel in terms of flavor retention during manufacturing (for drying, see Table 4.1). As noted earlier, modified food starches are excellent emulsifiers and emulsion quality has a strong influence on flavor retention during spray drying (Risch and Reineccius, 1986; Baranauskiene *et al.* 2007; Soottitantawat *et al.* 2003). Additionally, modified food starches can typically be used at 50–55% solids levels versus 30–35 % solids for gum acacia. Thus, although both gum acacia and modified food starches will yield good emulsions—meaning, they should yield good flavor retention—modified food starches yield the best flavor retention: partially because they can be used at higher in-feed solids levels, which also improves flavor retention (Reineccius, 1989). Maltodextrins, corn syrup solids or the simple sugars (and their alcohols) typically can be used at high infeed solids levels, but their poor emulsification properties result in poor flavor retention during drying.

A few authors have reported on the use of proteins for flavor encapsulation. For example, Kim and Morr (1996) evaluated sodium caseinate, whey protein isolate, soy protein isolate and gum acacia (control) for the encapsulation (spray drying) of orange oil. They found the soy protein isolate to be best at retaining orange oil during spray drying (85.7% retention), followed by sodium caseinate (81.5%), and gum acacia (75.9%), with whey protein isolate being the poorest (72.7%). One must note that the authors chose to use all materials at 10% solids and began by making an aqueous solution of each carrier at this concentration, then added orange oil at 10, 20 and 30% of the carrier solids. While this choice allows a comparison of the inherent ability of the carrier to retain volatile substances, it is an impractical approach from a commercial standpoint. Manufacturers use carriers that permit adequate atomization at the maximum solids content. Thus they would use each of these carriers at different solids levels based on the viscosity of the infeed slurry. Since solids content of the infeed slurry is the major determinant of flavor retention during drying, one would get a totally different ranking of carriers and retention if the experimental design involved using the carriers at constant viscosity.

Dronen (2004) reported on a study where she evaluated the retention of a volatile model flavor system (*water soluble components*) during spray drying prepared on a constant viscosity basis consistent with in-

dustry practice. She used 10 DE maltodextrin (MD) at 40% infeed solids (IS), 25 DE corn syrup solids (CSS) at 40% IS; gum acacia (GA) at 35% IS; modified starch (MS) at 45% IS, soy protein concentrate (SPC) at 15% IS and whey protein isolate (WPI) at 30% IS. These solids levels corresponded to ca. equivalent viscosities, which were low enough to permit atomization during spray drying (ca. 250 cp). She found the 10 DE MD to give 55% retention [R] ~ equal to GA (55% R) ~ equal to MS (51% R) but > 25 DE CSS (40% R), > WPI (25% R), and > SPC (13% R). Charve and Reineccius (2009) have provided a more detailed study, comparing proteins to the most common carbohydrate flavor encapsulants. Their findings on the ability to retain a model flavoring during drying were: gum acacia (94%), modified starch (88%), whey protein isolate (87%), soy protein isolate (77%), and sodium caseinate (65%). This order is different from Dronen's, since Dronen used a water soluble flavoring and Charve and Reineccius used a fat soluble model flavoring. This effect was most prominent in that the maltodextrin performed well for Dronen, since no emulsifying properties were required for her flavoring, while emulsification was critical for Charve and Reineccius. It is relevant that using formulations consistent with industry practice (varying solids based on carrier type), the order of flavor retention reported by both Dronen and by Charve and Reineccius was quite different from that reported by Kim and Morr (1996).

4.2.4.2. Retention During Storage

Flavoring materials may be lost during storage due to chemical reaction (with oxygen, the encapsulation matrix, or within the flavoring itself), or to diffusional losses to the environment. Let's first consider losses due to oxidation.

Any flavoring containing citrus oils is susceptible to oxidative reactions and the subsequent development of off-flavors. Thus, an important function of an encapsulation material is the protection of flavoring from oxygen. The materials used for flavor encapsulation differ greatly in this regard. The starch hydrolysates provide varying protection depending upon their dextrose equivalent (Anandaraman and Reineccius, 1986). In general, the higher the DE, the better the protection. This presents a problem, however, since higher DE materials tend to be difficult to dry, yield poor flavor retention, and are very hygroscopic. Thus, one can get excellent shelf-life using a high DE maltodextrin (more

correctly, a corn syrup solid) but have an otherwise unsuitable product. This observation also holds true for the use of mono- and disaccharides for encapsulation.

The observation that high DE starch hydrolysates (corn syrup solids) offered greater oxidative stability during storage has led to a number of commercial products that are blends of emulsifying wall materials and higher DE maltodextrins, corn syrup solids or simple sugars. Sufficient emulsifying wall materials are used to impart the necessary emulsifying capacity, and then the high DE maltodextrins, corn syrup solids or simple sugars are added to impart an oxygen barrier. One might use as little as 20% of an emulsifying wall material and 80% of a wall material that imparts oxidation stability. Fortunately, the starch hydrolysates are very inexpensive.

While the modified food starches produce an encapsulated flavoring which has excellent flavor retention and emulsion stability, they have traditionally provided very poor protection against oxidation (Reineccius, 1991; Krishnan *et al.* 2005). As new generations of modified starch have been developed, their performance in this respect has improved (for example, Hi-Cap100, Ingredion Incorporated.). If one compares the two parts of Figure 4.4, one can see that there is little difference between the oxidation profile of the gum acacia:maltodextrin blend (left) and Hi-Cap100 samples (right). However, one should recognize that gum acacia is a natural product, and that its oxygen barrier properties may differ greatly from one lot to another, as explained later.

The data in Figure 4.4 also give the reader an idea of the importance of RH on the oxidative stability imparted by these two materials. Interestingly, the least stable RH for both materials is 51% (evaluated 23, 51, 75 and 96% RH). For the Hi-Cap samples, 96% RH is the next least stable, and there is no significant difference between the 23 and 75% RH. For the gum acacia:maltodextrin samples, there was little difference in stability when the samples were stored at 23, 75 or 96% RH. There is substantial information in the literature suggesting that the ability of a given wall material to protect a sample against oxidation is not easily predicted based on structure or water activity.

Acacia products vary greatly in their suitability as encapsulating materials: there are several hundred different species of acacia trees, many of which yield a gum that may inadvertently enter commercial products. Thus, dilution of quality gums, or other natural variability in the preferred species, may result in variable stability when using this product. One will find some gums that offer excellent protection against
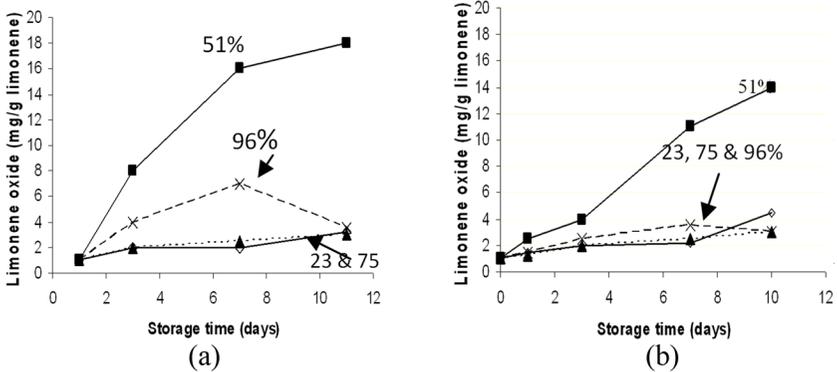
FIGURE 4.4. *Rate of limonene oxidation as influenced by RH of storage (50°C). (a) gum acacia:MD blend (1:3); (b) Hi-Cap™:MD blend (1:3). (Adapted with permission from Soottitantawat et al., J. Agric. Food Chem., 52, 1274. Copyright 200a American Chemical Society).*

oxidation, while another sample offers little. The plots shown in Figure 4.5 illustrate how stability varies with gum type (comparison of gum acacia 1 and 2). Gum acacia 2 affords much more stability to the encapsulated orange oil than gum acacia 1. The other two lines point out the importance of adding maltodextrins to gum acacia to make a lower cost carrier system. Gum acacia 4, with added maltodextrin, provided
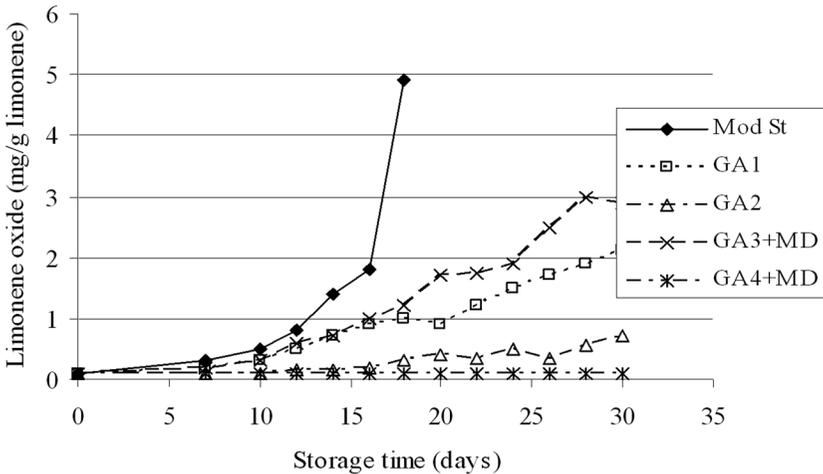
FIGURE 4.5. *Limonene oxidation when spray dried in different commercial encapsulating matrices (GA = gum acacia; GA+MD is a GA plus a maltodextrin; Mod St is a modified starch) and stored at 37°C. (numbers after GA in legend specify different gum acacias; Reprinted with permission from Allured Publishing, copyright July/August 1990; Risch and Reineccius, 1990).*

excellent stability to orange oil during storage at a lower cost than pure gum acacia.

There are a few publications on the effectiveness of proteins to pro- tect flavorings from oxidation. Of these, Kim and Morr (1996) spray dried orange oil in different proteins (Whey Protein Isolate (WPI), Soy Protein Isolate (SPI) and Sodium Caseinate (SC)), as well as in gum acacia (control), stored the resultant powders, and monitored oxidation in each. They found the WPI and SPI offered the best protection to the orange oil and the gum acacia and SC the poorest. Recent work by Charve and Reineccius (2009) comparing three proteins to traditional carbohydrate wall materials provided some additional information on the ability of proteins to protect citrus oils (limonene) against oxidation (Figure 4.6). It is obvious that all of the proteins were more effective at protecting the limonene from oxidation during storage than either of the carbohydrate materials (lower amounts of limonene oxide produced). A second observation drawn from Figure 4.6 is that materials spray dried at lower infeed solids were also less stable against oxidation than
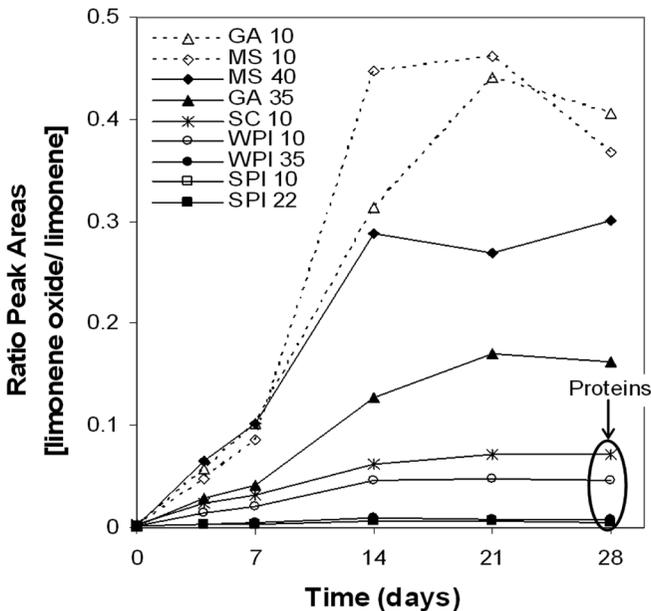
FIGURE 4.6. Limonene oxidation during storage of spray dried powders with different wall materials and infeed solids contents. GA: gum acacia; MS: Modified starch; WPI: whey protein isolate; SPI: Soy protein isolate; SC: sodium caseinate. The number after the abbreviation refers to the carrier solids when spray drying. (Reprinted with permis- sion from Charve J and Reineccius, GA, J. Agric. Food Chem., 57, 2490. Copyright 2009 American Chemical Society). 2009).

those spray dried at higher infeed solids. This suggests that lower infeed solids result in a more permeable structure than those formed when drying higher infeed solids emulsions. Unfortunately, the authors did not have time to determine why the powder produced at lower infeed solids afforded poorer protection to the encapsulated limonene. One might suggest that this is due to more entrapped air in the powder particle, or a less dense wall, thereby offering less resistance to oxygen migration.

There is a great deal more recent information on using proteins as encapsulation materials to protect lipids from oxidation. Of these papers, most have shown proteins to be very effective for this purpose (e.g. Rosenberg and Moreau 1996; Jimenez *et al.* 2004; Robert *et al.* 2003; Bylaite *et al.* 2001; Wang *et al.* 2011). Rosenberg has postulated that protection derives from the protein moving to the lipid:carrier interface and forming an oxygen impermeable membrane. An interesting article by Bao *et al.*, (2011) noted that oxidative stability was increased by enzymatic crosslinking of the protein wall material. They postulated that an optimum level of cross linking further limited oxygen permeability of the capsule wall.

One of the major advantages of cyclodextrins is their ability to protect their guests from oxidation (Szente and Szejtli 2004). This capability is so well known that it has become common knowledge. While there is much less information on the stabilizing properties of inclusion complexes formed between volatiles and amylose, it appears that these complexes are also stable to oxidation (e.g. Wuff *et al.* 2005). A review by Conde-Petit *et al.* (2006) and a recent article by Ades *et al.*, (2012) provide overviews of this process.

The idea of simply plating a flavor (dry blending it onto a carrier) is very attractive from a cost standpoint. This process is used in some flavor applications, with a silica serving as the carrier (e.g. Bolton and Reineccius, 1991; Corkery *et al.*, 2011), or with microbial cells (Round and Nelson, 2006; Nelson, 2005). There has also been some work on plating flavorings onto carbohydrates such as porous starches (Bolton and Reineccius, 1991; Belingheri *et al.*, 2011; Buttery *et al.*, 1999; Glenn *et al.*, 2010).

Belingheri *et al.* (2011) compared the performance of blending diacetyl (0.5%) diluted in different solvents (ethanol, propylene glycol, triacetin and medium chain triglycerides) on to Starrier™ (Cargill), to spray drying diacetyl in a blend of gum acacia and maltodextrin. They monitored the retention of diacetyl during manufacturing and storage. Unfortunately, they did not define the storage conditions, whether open
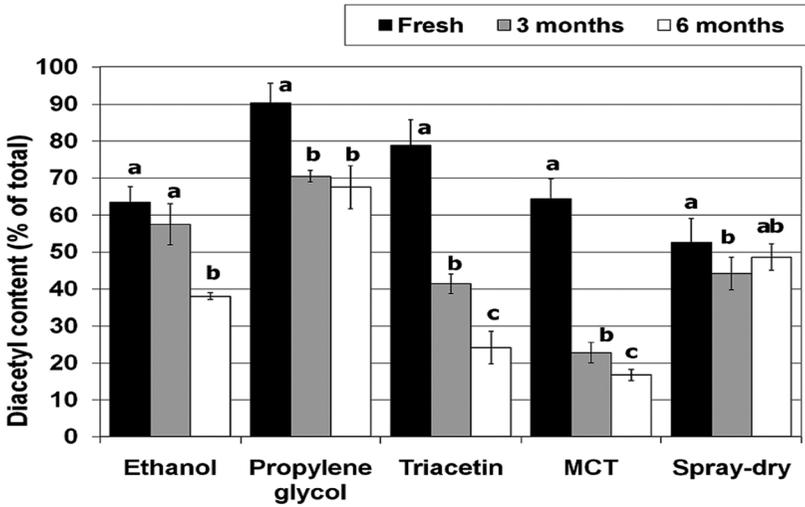
FIGURE 4.7. Diacetyl content of porous starch and spray dried sample during manufacture (fresh) storage (3 and 6 months) (Reproduced from Belingheri et al., 2011, Food Funct., 3, 257 with permission of The Royal Society of Chemistry).

to the atmosphere or closed to storage temperature/humidity. It is interesting that the diacetyl plated in propylene glycol was better than the spray dried or other plated samples (Figure 4.7). Good retention of diacetyl in a process that would not give any true encapsulation was attributed to a bonding of the polar diacetyl to the polar starch carrier. Their choice of volatiles to study was interesting, in that diacetyl tend to diffuse out of all carrier materials even when effectively encapsulated. High polarity and small molecular weight make its retention during storage problematic.

There is little information in the public domain on the ability of co-acervate systems to protect flavorings against oxidation. The few papers on this topic are focused on the preservation of highly oxidizable oils (fish, algal). For example, Barrow et al. (2007) commented on the stability of highly unsaturated oils (fish, algal, etc.) when coacervated using their proprietary method of encapsulation. While no data were provided in this article, they claimed stability for 18 months, while achieving a 60% load. Yan (2005) described his product and provided some data on storage stability. The coacervates Yan worked with were based on gelatin (Type A), with either polyphosphate or gum acacia. Yan used an accelerated oxidation test to evaluate stability of different coacervate formulations (all containing antioxidant). He reported induction periods of 26–38 hrs at 65°C, depending upon the formulation.

Unfortunately no controls or competing encapsulation techniques were provided for comparison purposes. Lamprecht *et al.* (2001) have also provided some information on the stability of coacervate systems. They worked with a gelatin:gum acacia system and omega 3 fatty acid esters. Their work focused on evaluating how the means of hardening (drying) coacervation systems compared with respect to oxidation. They provided data showing that coacervation resulted in significant protection to the fish oil compared to non-encapsulated systems; but again no data were provided relative to other encapsulation systems. More recently, Liu *et al.* (2010) have presented a formulation (gelatin:gum acacia) coacervation process and storage data on encapsulating flaxseed oil. As is seen in Figure 4.8, the peroxide value was more stable over time than unencapsulated flaxseed oil. Unfortunately, the authors did not study any reference encapsulates to determine the efficacy of this coacervation process vs. other, traditional processes, such as spray drying.

4.2.5. Flavor Release

Flavor release can have several meanings. As used in this chapter, it will refer to how an encapsulated flavoring gives up its contents. A
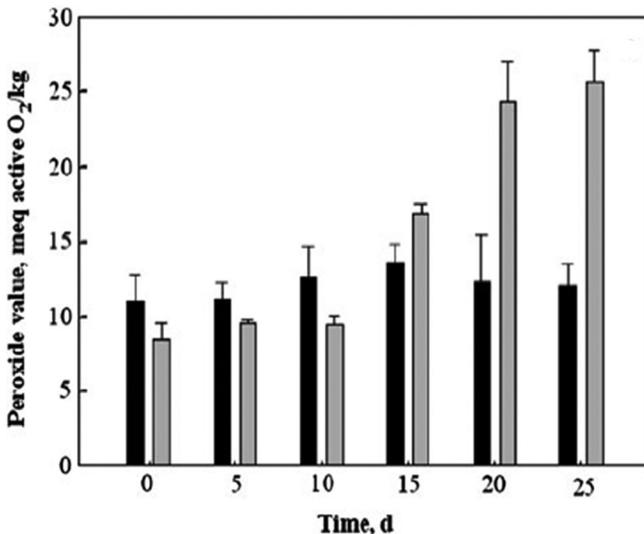
FIGURE 4.8. *Changes to peroxide values for bulk and entrapped flaxseed oil under ambient storage conditions. Data represent the means ± one standard deviation of duplicate batches (three replicates per batch) (With kind permission from Springer+Business Media: J American Oil Chemists' Soc., Entrapment of flaxseed oil within gelatin-gum arabic capsules, 87, 2010, 814, Liu S, Low NH, Nickerson MT, Figure 3).*

shortcoming of the major process used for flavor encapsulation (spray drying) is its inability to use edible films that are water insoluble or, minimally, slowly water soluble. The spray drying process requires that an aqueous soluble, edible film must be used in manufacturing, which means that all products of this process release flavor upon contact with water.

While in a sense all encapsulated flavorings offer controlled release (release only after contact with water), one can appreciate that additional controlled release properties may be desirable in certain product applications. For example, one may desire that an encapsulated flavoring does not release its contents during early stages of thermal processing. A delayed release may result in less flavor loss since the flavor would be protected from the heat until late in processing. Here a slow or delayed release is desirable. In the case of an encapsulated flavoring for a dry beverage mix, one desires a rapid release on reconstitution. Thus, the desired flavor release will be dependent upon the application. Currently, these controlled release properties can be attained directly through the use of coacervation and, potentially, extrusion and inclusion complex formation. Since spray dried particles are water soluble, controlled release properties may be imparted to them only through the application of secondary coatings: coating with a fat or shellac. Because secondary coatings (fats, oils, and shellacs) are costly and problematic to apply, it is desirable to accomplish controlled release by choosing the appropriate encapsulation technique. The topic of controlled release is deserving of its own chapter, so the following discussion will be overview in nature.

As mentioned above, coacervation, extrusion and cyclodextrins all may be used to impart controlled release properties to a flavoring. Coacervation does this through the use of capsule materials that may be insoluble in a final application. Cross linked capsules will be insoluble irrespective of the food system, while non-crosslinked capsules may be "soluble" or insoluble depending upon the sample environment. If the capsules are insoluble and retain their integrity in a food application, release is governed by diffusion through the capsule wall. For example, Yeo *et al.* (2005) provided information on the controlled release of a flavor oil prepared by complex coacervation. They reported on how formulation, freeze thaw cycles, and ionic strength will affect the release of an encapsulated oil. In essence, the dissolution of the capsule (in an ionic solution) resulted in rapid release, while systems where the wall matrix remained intact gave slower, diffusion controlled release.

Weinbrecht *et al.* (2004) reported on the encapsulation of sunflower oil, lemon oil and orange oil in a gum acacia:whey protein coacervate. They found that larger capsules gave the strongest flavour release (in eating). The glutaraldehyde cross-linked batches (small and large capsules) gave the lowest release intensity.

Controlled release properties may be imparted to flavorings encapsulated by extrusion processes by using some portion of the wall material which is somewhat insoluble. This approach is illustrated in a patent issued to McIver *et al.* (2002), where they included agar (7%) in a typical extrusion formulation. They produced an extruded capsule initially containing 13.6% cinnamic aldehyde. This capsule still contained 13.1% cinnamic aldehyde after being in water for 4 days.

Cyclodextrins tend to produce a controlled release products since they must be hydrated to release any flavor; and then the extent of release is based on partitioning of the guest between the food system and the cyclodextrin cavity. As one would expect, this is based on the affinity of the host for the guest. As an example, Reineccius *et al.* (2003) demonstrated that as little as 10% of menthol is released from β-cyclodextrin when placed in an aqueous solution, but more than 70% of ethyl butyrate is released under similar conditions. Mourtzinos *et al.* (2008) demonstrated a similar disparity in release of geraniol and thymol from β-cyclodextrin: geraniol was rapidly released (nearly 100%) while thymol was only slowly and incompletely released: 30% into aqueous solution.

4.3. CARBOHYDRATES USED IN FLAVOR DELIVERY

Of the materials used in flavor delivery, water-soluble carbohydrates are the most widely used. These carbohydrates serve different functions in different delivery systems. The most commonly used carbohydrates are listed in Table 4.1. Proteins have found very minimal use except in the coacervation process. These and some relatively new alternative edible encapsulating materials will be discussed individually.

4.3.1. Gum Acacia

Gum acacia (or gum arabic) is the material traditionally used for the encapsulation of food flavors. It is derived from the gum acacia tree, which is grown in the semi-desert region of North Central Africa (Thevenet, 1986). While there are over 1,350 species of the acacia tree

Analytical Methods for Carbohydrate Texturizing and Delivery Systems

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7.1. INTRODUCTION

EVALUATION of carbohydrate texturizing and delivery systems, and of carbohydrate ingredients used in these systems, entail sophisticated analytical techniques if structure is to be correlated with functional properties. In addition, analyses of the final products are essential to determine quality and stability. This chapter will discuss analytical methods for characterization of carbohydrates, and physicochemical characterization of the delivery systems, including important properties of emulsions and solid products.

7.2. CHEMICAL CHARACTERIZATION OF CARBOHYDRATES

Several analytical techniques are used to characterize carbohydrates. Critical parameters include analysis of sugar components, dextrose equivalent for starch and starch products, degree of polymerization, molecular weight distribution, the degree of branching and of substitution. These properties affect the functional properties of carbohydrates and their performance as emulsifiers, stabilizers or texturizers in food systems.

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Sugar components of polysaccharides are determined primarily by chromatography. High performance liquid chromatography (HPLC) is the most commonly used method to elucidate sugar composition of carbohydrates and polysaccharides. Gas chromatography (GC) is also employed after derivatization of sugars to alditols, but this is a time consuming method. HPLC is the method of choice for the analysis of carbohydrates. Before analysis, the sample requires pretreatment, such as fat extraction, and removal of salts or proteins. The defatted sample then undergoes hydrolysis using an acid and/or enzymes. After neutralization and/or inactivation of enzymes, the sample containing the sugars and some residual low molecular weight carbohydrates is cleaned and filtered, then injected into an HPLC equipped with an appropriate column and a differential refractive index detector. Standard solutions with known concentrations, such as arabinose, glucose, galactose, mannose, and xylose, are analyzed at the same time. In some instances, sugars cannot be resolved or separated completely using the HPLC. Agblevor *et al.* (2007) formulated a method in which, by careful manipulation of the HPLC mobile phase, they were able to separate and analyze quantitatively biomass monomeric sugars (arabinose, xylose, fructose, glucose, mannose and galactose), as well as dimeric sugars (cellobiose, sucrose). Biomass sugars are typically difficult to analyze due to the presence of many other components that can interfere with the analysis. Raessler *et al.* (2008) formulated an easy method that allows the isocratic chromatographic determination of 12 carbohydrates and sugar alcohols from one sample within 30 min (Figure 7.1). Separation and quantitation of simple carbohydrates was based on isocratic high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD), using 18 mM NaOH as eluent (Raessler *et al.*, 2008). The method was used to determine non-structural carbohydrates in grasses. Yadhav *et al.* (2007) analyzed the sugars from corn fiber gum using HPLC equipped with pulsed amperometric detection (HPAEC-PAD) after methanolysis of sample combined with trifluroacetic acid hydrolysis. Sugar components can also be quantified using assay kits, such as those supplied by Megazyme International.

Another analytical parameter used by the industry to characterize starch products is the dextrose equivalent (DE). This is the reducing power of starch products expressed as percentage of reducing sugar as dextrose on a dry basis. Using dextrose as a reference, which has a DE of 100, starches and starch products have a DE ranging from 0 for starches, to DE of 73 or higher for corn syrups. DE for starches and
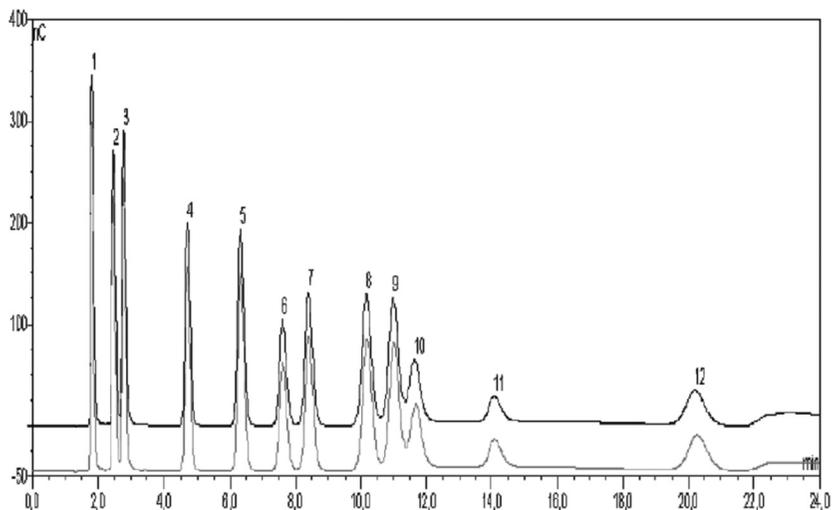
FIGURE 7.1. Analysis of the "standard mixture" including 12 carbohydrates and sugar alcohols; concentration = 10 mg.nL for each carbohydrates. Peaks: 1, inositol; 2, sorbitol; 3, mannitol; 4, fucose; 5, salicin; 6, rhamnose; 7, arabinose; 8, galactose; 9, glucose; 10, sucrose; 11, fructose; 12, raffinose. Reprinted with permission from Raessler et al., J. Agric. Food Chem., 56, 7650. Copyright 2008 American Chemical Society.

starch products is an indication of the degree of hydrolysis. It is used to describe the extent of hydrolysis of starch hydrolyzates, such as malto-dextrins. The methods employed to determine the DE of starch products include the Lane-Eynon titration method and osmometry. The Lane-Eynon method determines the reducing power of carbohydrates through titration, using copper sulfate in an alkaline solution. This method can produce consistent results if handled by an experienced analyst, because accurate reproducible results can only be obtained by following precise steps and attention to details, such as boiling time, volume of titrant. Determination of the DE through osmometry is done by measuring the freezing point depression of a carbohydrate solution. The DE is obtained from a calibration curve of freezing point depression versus DE obtained through the Lane-Eynon method. This calibration chart is prepared from solutions with different known DEs and known freezing point depression. Because osmometry is faster and easier to use than the Lane-Eynon method, process control in starch manufacturing plants relies more on osmometry for DE determination.

The degree of polymerization (DP) is an important chemical property of carbohydrates, especially the high molecular weight carbohydrates, because DP affects their physicochemical and functional properties:

emulsifying efficiency, rheological properties. *DP* is the number of monomeric or base units in a polymer chain. It is equal to molecular weight divided by the molecular weight of one monomeric unit. Together with molecular weight distribution, average molecular weight and z-average radius of gyration (R_g), molecular characterization will provide an insight on the functionality and performance of carbohydrate matrices. To distinguish the different terms used to describe high molecular weight carbohydrates, a few definitions are in order. Molar mass (*M*) is defined as the mass of 1 mole of polymer (gmol^{-1} or kgmol^{-1}). The molar mass of a homopolymer is related to the DP by this simple relationship: $M = DP \times M_o$ (M_o = molar mass of the repeating unit). A polymer is a mixture of molecules with different degrees of polymerization. The number-average molar mass (M_n) is the sum of the products of the molar mass of each fraction multiplied by its mole fraction. The weight fraction (W_i), on the other hand, is defined as the mass of molecules of molar mass, Mi, divided by the total mass of all the molecules present. The weight-average molar mass (M_w) is the sum of the products of the molar mass of each fraction, multiplied by its weight fraction. M_w is used more often than M_n for carbohydrate polymers, because the physical properties, and consequently the functional properties, are affected by high molar mass (M_i) fraction. The radius of gyration R_g is defined as the root mean square distance of the collection of atoms from their common center of gravity. R_g depends on the solvent used to solubilized the polymer. In a poor solvent, R_g of a polymer is smaller than it is in a good solvent. If the molecule is branched, its R_g is smaller compared to a molecule without branching, even if the molecular weights are the same. In order to better understand solution behavior, rheology, molecular interaction of carbohydrates (both intra and intermolecular interaction) and functional properties, knowledge of size, structure and conformation of carbohydrates is very important.

Characterization of the size and molecular distribution of starch products and polysaccharides is accomplished through size exclusion chromatography (SEC), also known as gel permeation chromatography (GPC). Three types of detectors are used in SEC: differential refractive index (DRI), multiple-angle light scattering (MALLS) and in-line viscometry detectors. Several factors need to be considered in getting the right measurement in characterizing size and molecular weight distribution of starches and polysaccharides: (1) complete dissolution without degradation of the sample; (2) complete separation of components; (3) use of appropriate concentration for analysis; (4) use of the right

solvent for complete dissolution of sample; and (5) employment of dissolution enhancement techniques, such as thermal (heating, microwaving) or mechanical treatment (shaking, sonication) without degradation of the molecule.

White (1999) employed gel permeation chromatography equipped with MALLS and Optilab interferometric refractive index detectors (both from Wyatt Technology, Inc., Santa Barbara, CA) to characterize selected industrial polysaccharides: native and modified starches, dextrins, dextrans, glucans, pullulans, modified celluloses, pectins, carrageenans, and gums from microbial and plant seed sources. The HPLC system used was a Waters (Milford, MA) 2690 solvent delivery system, which includes a built-in degasser, a thermostatted sample compartment, injector, and a column oven similar to that in Figure 7.2. The columns used were Water Ultrahydrogel 2000 and 250 in series, thermostatted to 45°C; and the mobile phase used was either 0.025 M phosphate buffer with added 0.05 M NaCl, or 0.1 M LiCl for charged polysaccharides, such as carrageenans, to inhibit gel formation (White, 1999). Samples were prepared by dissolving the polysaccharide in the mobile phase to obtain a concentration from 1–3 mg mL^{-1}.

Figure 7.3 shows the GPC-LS chromatograms of four gums characterized in this study. It can be clearly determined that xanthan gum is the largest polymer, followed by guar, then κ-carrageenan, with gellan

FIGURE 7.2. A Waters HPLC equipped with MALLS and DRI detectors.
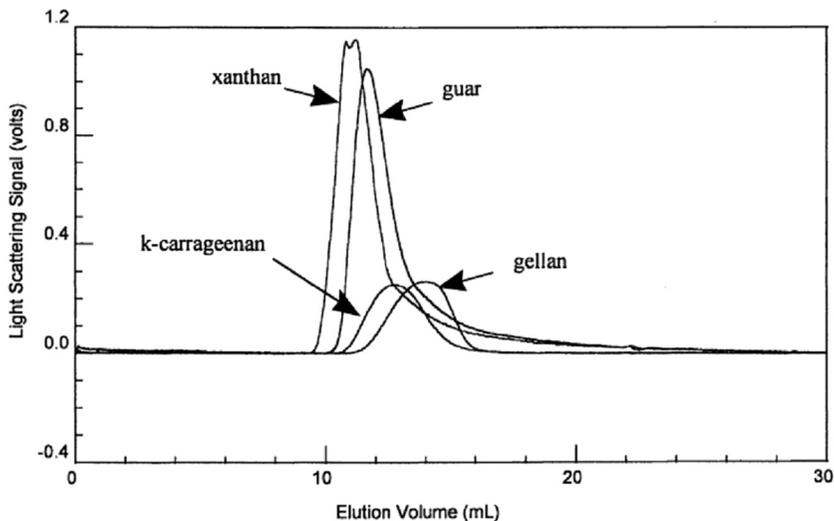
FIGURE 7.3. GPC-LS overlay of four commercial gums. Reprinted with permission from White, Applications of Gel Permeation Chromatography with Multi-Angle Light Scattering to the Characterization of Polysaccharides. In: El-Nokaly MA and Soini HA (Eds.) Polysaccharide Applications, p. 311. Copyright 1999 American Chemical Society.

the smallest among the four (White, 1999). Table 7.1 summarizes the M_w and R_g of the four gums based on the GPC analysis. According to White (1999), the M_w for guar was lower than expected, and both its Mw and the radius were about half that of xanthan, while the results for gellan and κ-carrageenan were very much in line with expected results.

Figure 7.4 and Table 7.2 present the GPC results for potato amylopectin and soluble potato starch samples, showing the monodispersity

TABLE 7.1. Summary of M_w and R_g.

Gum	M_w (x 10^{-6}g mol^{-1}) (lit.)*	M_w/M_n (lit.)*	d_n/d_c (mL/g)	$R_{g,z}$ (nm) (lit.)*
Xanthan	4.87 ± 2.8 (2.2, 7)	1.06	0.414	228 ± 15
Guar	1.98 ± 2	1.16	0.14	113 ± 3
Gellan	0.298 ± 0.02 (0.15–0.56)	1.58	0.133	70 ± 5
κ-carrageenan	0.495 ± 0.03 (0.468), (0.34–0.58), (0.42)	1.53	0.115	73 ± 3 (60)

*lit. - literature value

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of the amylopectin relative to the starch (White, 1999). The average slope for purified amylopectin is 0.35, which is expected for highly branched molecules, while for the starch, the slope is about 0.50, due to the presence and contribution of amylose, which is basically a linear molecule. White (1999) has shown that some basic light-scattering principles and GPC can be applied to a variety of polysaccharides for the estimation of absolute molecular weight and the radius of polysaccharides, without the need for column calibration methods or standards. The technique is suitable for characterizing a wide variety of industrial polysaccharides, and the results obtained are of comparable accuracy to those obtained by other methods. GPC-MALLS is a useful tool for the characterization and quality control of polysaccharides of industrial importance, particularly where functional properties are dependent on molecular size or conformation (White, 1999).

Gillespie and Hammons (1999) employed SEC using three detectors (DRI, viscometer, MALLS) coupled to a standard SEC/GPC (or SEC^3). The viscometer measures intrinsic viscosity of the polysaccharide. In
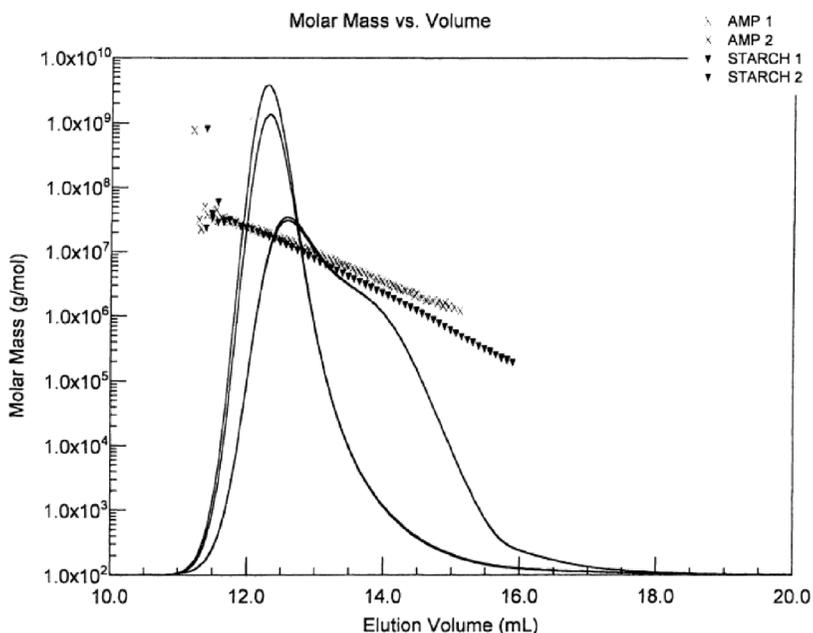
FIGURE 7.4. GPC-LS overlay of commercial potato amylopectin (X) and soluble potato starch (▼) with molar mass superimposed. Reprinted with permission from White, Applications of Gel Permeation Chromatography with Multi-Angle Light Scattering to the Characterization of Polysaccharides. El-Nokaly MA and Soini HA (Eds.) Polysaccharide Applications, p. 313. Copyright 1999 American Chemical Society.

TABLE 7.2. Summary of M_w and R_g. Results for Replicate Analysis of Potato Amylopectin and Soluble Potato Starch.

Gum	M_w (x 10^{-6}g mol^{-1})	M_w/M_n	d_n/d_c (mL/g)	$R_{g,z}$ (nm)
Amylopectin (run1)	10.48	*2.24*	0.146	58
Amylopectin (run2)	10.04	*2.45*	0.146	57
Starch (run 1)	2.30	*3.46*	0.146	39
Starch (run 1)	2.47	*3.25*	0.146	39

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this study, the use of MALLS measured molecular weight directly, without the use of calibrants; and selected polysaccharides (dextran, maltodextrin, starch, carrageenan, hyaluronic acid and chitosan) were analyzed using the SEC. Figures 7.5–7.6 show the chromatograms of maltodextrin and high amylose starch which have very large polydispersities. This is also illustrated in Figure 7.7, which shows the overlay chromatograms of corn syrup solids, maltodextrin, starch and dextran. This also clearly shows the molecular differences between these 4 samples. The SEC[3] Mark-Houwink structural comparison is given in Figure 7.8, ranking the samples from lowest to highest in terms of the amount of branching at the same molecular weight in this order: dextran, high amylose starch, maltodextrin and corn syrup solids (Gillespie and Hammons, 1999). According to Gillespie and Hammons, SEC[3] is a powerful
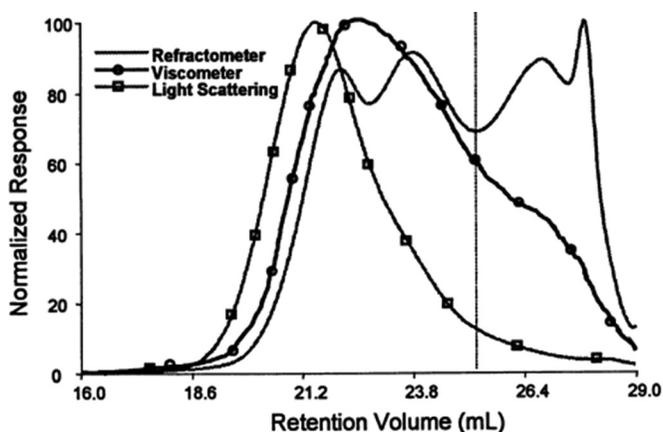
FIGURE 7.5. SEC[3] chromatogram of maltodextrin. Reprinted with permission from Gillespie and Hammons, Analysis of Polysaccharides by SEC. In: Provder T (Ed.) Chromatography of Polymers, 293. Copyright 1999 American Chemical Society.
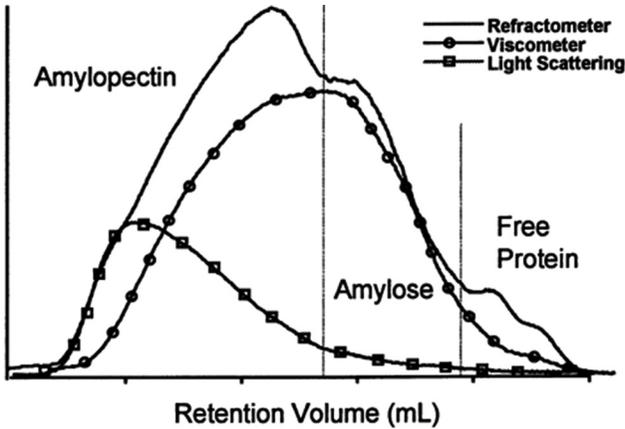
FIGURE 7.6. SEC^3 chromatogram of high amylose starch. Reprinted with permission from Gillespie and Hammons, Analysis of Polysaccharides by SEC. In: Provder T (Ed.) Chromatography of Polymers, 294. Copyright 1999 American Chemical Society.

technique for determining the molecular weight and the microstructure of polysaccharides, and with the addition of the viscometer detector, it allows measurement of differences due to sample preparation techniques and running conditions.

Studies on the use of HPSEC-MALLS-RI in molecular characterization of corn starch, and in the characterization of amylose in terms of molecular weight, weight- and molar-based distributions of degree of polymerization, and fine-structure of amylopectin, were undertaken by You and Lim (2000) and Chen and Bergman (2007), respectively.
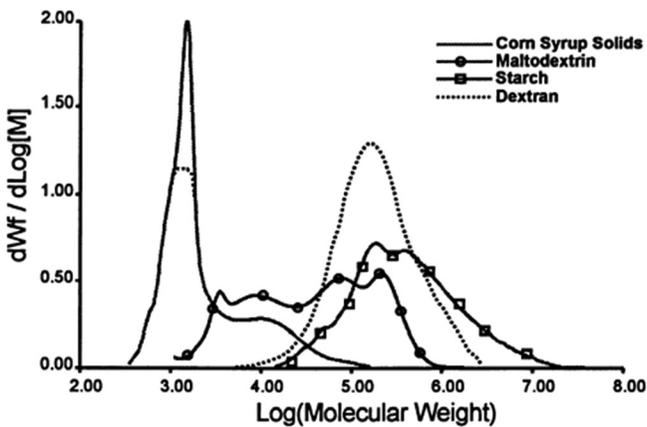
FIGURE 7.7. Wide ranging molecular weight distribution overlay. Reprinted with permission from Gillespie and Hammons, Analysis of Polysaccharides by SEC. In: Provder T (Ed.) Chromatography of Polymers, 295. Copyright 1999 American Chemical Society.
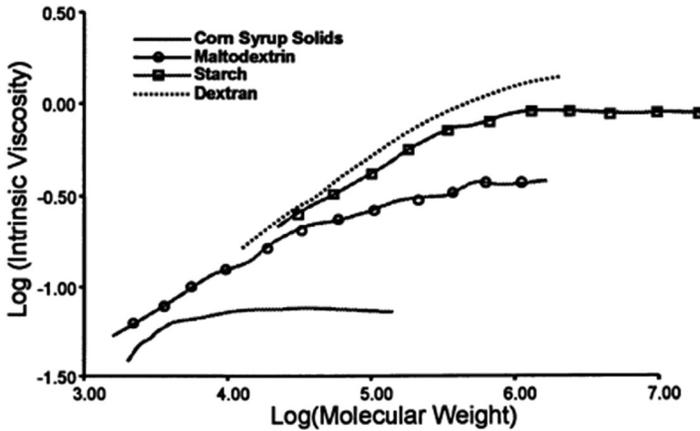
FIGURE 7.8. SEC³ Mark-Houwink structural comparison. Reprinted with permission from Gillespie and Hammons, Analysis of Polysaccharides by SEC. In: Provder T (Ed.) Chromatography of Polymers, 295. Copyright 1999 American Chemical Society.

Diaz *et al.* (2009) employed high performance size-exclusion chromatography with multi-angle laser light scattering and refractive index detectors (HPSEC-MALLS-RI) to determine the weight average molecular weight (M_w) and root mean-square (rms) radius or R_g of the polymers in the tomato serum. With this powerful tool, they were able to determine that the Mw changed from 2.62×10^5 g/mol in the juice to 2.61×10^5 g/mol in the paste, indicating minimal depolymerization oc-
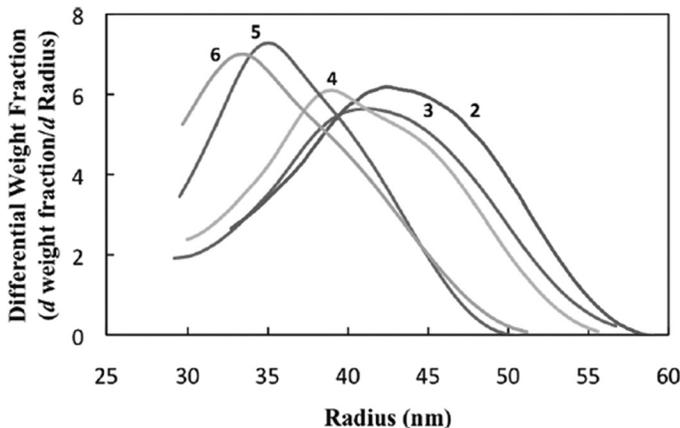
FIGURE 7.9. Changes in the rms radius of serum pectin obtained during various steps in tomato juice concentration: sample/where sampled (1) whole tomato; (2) flash evaporator; (3) third-effect evaporator; (4) second-effect evaporator; (5) first-effect evaporator; (6) paste. Reprinted with permission from Diaz et al., J. Agric. Food Chem., 57, 8456. Copyright 2009 American Chemical Society.

curred; but the rms radius distribution indicated that the pectin conformation became more compact as the juice became more concentrated (Figure 7.9). According to Diaz *et al.* (2009), in processing intermediates taken from later stages in the process and in the paste, the shape factor changed to about 0.25, indicating a more compact conformation; and this conformational change correlated with the observed decrease in serum viscosity in the paste production process (Figure 7.10).

Liang *et al.* (2013) studied the stability and bioaccessibility of β-carotene in nanoemulsions stabilized by modified starches (MS). In this study, they characterized the commercial n-OSA modified starches using a HPSEC-MALLS-RI system—an HP 1050 series pump and an autoinjector valve (Hewlett-Packard, Valley Forge, PA) with a 100 μL injection loop and MALLS detector (Dawn DSP-F, Wyatt Technology, Santa Barbara, CA) with a He–Ne laser source ($k = 632.8$ nm), a K-5 flow cell, and an RI detector (model ERC-7512, ERMA Inc., Tokyo, Japan), and with two aqueous SEC columns (Ultrahydrogel 250 and 1000, Millipore Co.), connected in series to determine the molecular weight distributions of the modified starches. Based on the results (Figure 7.11 and Table 7.3), the dispersed molecular density of MS could affect the droplet diameters, chemical stability and in vitro digestion of nanoemulsions (Liang *et al.*, 2013).

The degree of substitution (DS) is another critical property of poly-
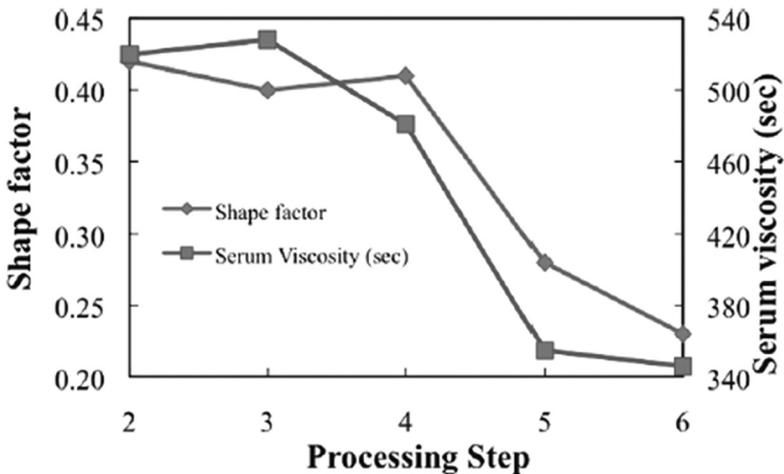
FIGURE 7.10. Relationship between serum viscosity and pectin conformation. Shape factor or scaling factor indicates the general conformation of the polymer, either extended or coiled. Reprinted with permission from Diaz et al., J. Agric. Food Chem., 57, 8456. Copyright 2009 American Chemical Society.
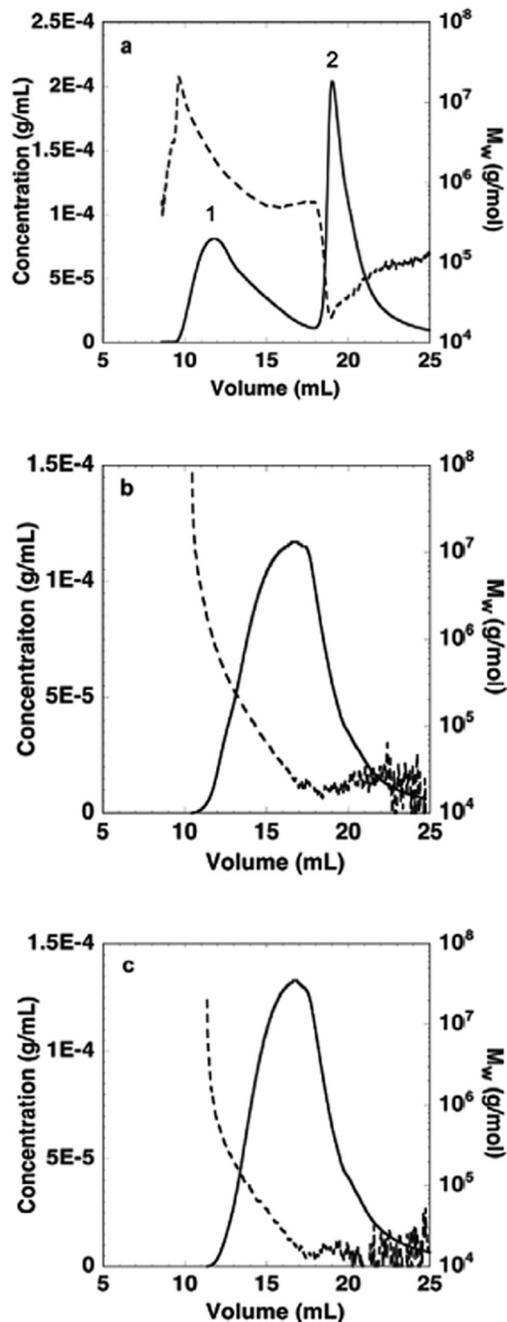
FIGURE 7.11. Weight-average molecular weight (Mw) and concentration signals vs elu-
tion volume profiles of MSs A (a), B (b), and C (c), determined by an HPSEC-MALLS-RI
system: a solid line denotes the concentration signal, and a dashed line the Mw distribu-
tion. Reprinted with permission from Liang et al., J. Agric. Food Chem., 61, 1252. Copy-
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TABLE 7.3. Weight-Average Molecular Weight (Mw), z-Average Radius of Gyration (Rz), and Dispersed Molecular Density (ρ) of n_OSA-Modified Starches A, B, and Ca.

Modified Starch	peak 1 M_w (10^4 g/mol)	peak 2 M_w (10^4 g/mol)	av M_w (10^4 g/mol)	R_z (nm)	ρ (g/mol.nm³)
A Hi-Cap 100	206.6 ± 0.02	7.85 ± 0.01	94.2 ± 0.03	32.3 ± 0.1	28.0 ± 0.3[c]
B Capsul			8.29 ± 0.02	18.7 ± 0.5	12.7 ± 1.4[a]
C Capsul TA			4.02 ± 0.01	20.1 ± 1.3	20.1 ± 1.3[b]

[a]Data expressed as the mean ± SD (n =3). For ρ values, data followed by different online letter (i.e., a–c) are significantly different (P < 0.05). Reprinted with permission from Liang et al., J. Agric. Food Chem., 61, 1252. Copyright 2013 American Chemical Society.

saccharides, especially for the n-OSA esterified starches, because the emulsification efficiency is determined by the % n-OSA substitution. DS is the average number of hydroxyl groups that have been replaced by a n-OSA group. This can be determined by titrimetric method. The n-OSA modified starch is weighed and suspended in a standard KOH solution. This is shaken for 12 hours in an orbital shaker. Excess alkali is titrated with standard HCl solution and retitrated after 2 hours. Another method calls for using NaOH solution instead of KOH solution (Gracza, 1965). Song *et al.* (2006) described the method. An n-OSA starch sample (5 g, dry weight) is weighed accurately and dispersed by stirring for 30 min in 25 mL of 2.5 M HCl-isopropyl alcohol solution. Added to this is 100 mL of 90% (v/v) aqueous isopropyl alcohol solution, and the slurry is stirred for an additional 10 min. The suspension is filtered through a glass filter, the residue washed with 90% isopropyl alcohol solution until no chloride ions can be detected using 0.1 M $AgNO_3$ solution. The starch is re-dispersed in 300 mL distilled water, then cooked in a boiling water-bath for 20 min. The starch solution is titrated with 0.1 M standard NaOH solution, using phenolphthalein as an indicator, and a blank is simultaneously titrated. DS is calculated based on the following equation (Song *et al.*, 2006):

$$\frac{0.162 \times (A \times M)/W}{1 - [0.210 \times (A \times M)/W]}$$

where A is the titration volume of NaOH (mL), M is the molarity of NaOH solution, and W is the dry weight in grams of the n-OSA starch.

Tizzotti *et al.* (2011) developed a novel, fast and straightforward method for the characterization of starch and n-OSA-modified starches using ^{1}H NMR to measure the degree of branching (DB) and the degree of chemical substitution (DS). In brief, the starch/modified starch is dissolved in methyl-d_6 sulfoxide (d_6-DMSO) and a very small amount of deuterated trifluoroacetic acid (d_1-TFA) is added. This method utilizes the fast exchange between labile protons of hydroxyl groups and labile deuterons of d_1-TFA. According to Tizzotti *et al.*, this method provides a clear and well-defined ^{1}H NMR spectrum and gives an improved technique for determining the degrees of both branching and chemical substitution, with better accuracy than current techniques. Figure 7.12 shows the ^{1}H NMR spectrum of corn starch. Upon computation, the value found for the corn starch is DB = 3.34 ± 0.02% (n = 3), which is consistent with what is generally observed for starches since DB generally lies between 1 and 5%, depending on the botanical source and/or the amylose/amylopectin ratio (Tizzotti *et al.*, 2011). Likewise the DS based on the ^{1}H NMR spectrum of n-OSA modified corn starch (Figure 7.13) is 0.0326 ± 0.0006 (*n* = 3) which has good accuracy (Tizzotti *et al.*, 2011).
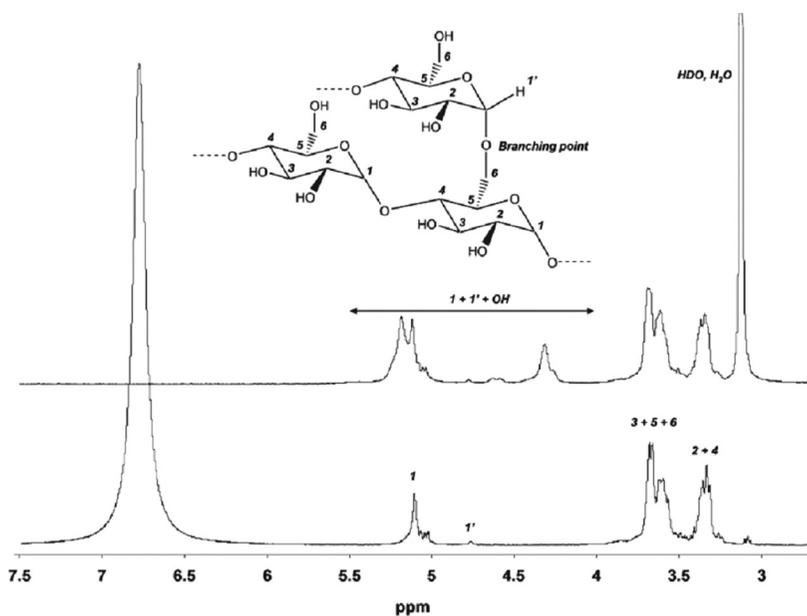
FIGURE 7.12. ^{1}H NMR spectrum of corn starch in d6-DMSO at 70°C prior (upper panel and after (lower panel) addition of d_1-TFA. Reprinted with permission from Tizzotti et al., J. Agric. Food Chem., 59, 6916. Copyright 2011 American Chemical Society.

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