

Lactose and the Sugars of Honey and Maple: Reactions, Properties, and Analysis

Landis W. Doner¹ and Kevin B. Hicks¹

LACTOSE

Introduction

Over the past ten years, there has been considerable interest in the preparation, reactions, properties, and analysis of lactose. Much of this interest has been stimulated by the worldwide need to utilize an abundant by-product, cheese whey, of which lactose is the major component. Several excellent reviews have been compiled on various aspects of lactose research, to which the reader will be referred in the appropriate section. The present review covers the most recent and significant research on the preparation and properties of lactose, its reactions to yield potential food carbohydrates, and the analytical methods useful in these research areas.

¹USDA-SEA-AR, Eastern Regional Research Center, Philadelphia, PA 19118.

Methods for Isolation of Lactose

Lactose, 4-*O*- β -D-galactopyranosyl-D-glucopyranose (Fig. 7.1), commonly called milk sugar, is the major carbohydrate present in the milk of most mammals. Excellent reviews are available on the mammary biosynthesis of lactose (Nickerson 1974; Hansen and Gitzelmann 1975) and the occurrence and levels of this sugar in milks from various species (Nickerson 1974).

The major commercial raw material from which lactose is isolated is whey, a by-product of cheese manufacture. For every pound of cheese produced, approximately 9 lb of whey are obtained (Thelwall 1980). Whey contains about 6% solids, of which approximately 4.7% is lactose, 0.7% protein, and 0.5% minerals (Harju and Kreula 1980). On a worldwide basis, it has been estimated that three to five million tons of lactose are available yearly, in the form of cheese whey. Due to low market prices and high manufacturing costs, much of this potential has not been realized. As recently as 1976 (Clark 1979), only about one-half of the whey in the United States was processed, with the other half disposed of by any available method. Increased restrictions on disposal of wastes with high biological and chemical oxygen demand now force cheese manufacturers to find new outlets for whey and the lactose it contains. Stimulated by these economic realities, researchers have developed new and more efficient methods for isolating lactose from whey. Traditional methods for lactose isolation have been reviewed by Nickerson (1974). These require energy intensive heating, filtration, and evaporation to remove residual protein and salts before the lactose is crystallized from concentrated solutions. Recently, however, methods have been developed for removal of the protein from whey by ultrafiltration (Matthews 1979). These largely deproteinated whey permeates make ideal sources from which lactose may be isolated. Delbeke (1979) showed that treatment of an ultrafiltration permeate with adsorption, strong acid, and weak base ion exchange resins yields an effluent containing 99.74% lactose on a total solids basis. This process effectively

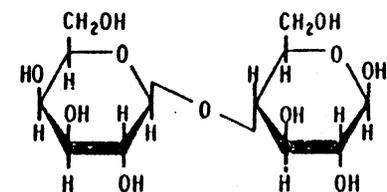


FIG. 7.1. STRUCTURAL FORMULA OF LACTOSE (4-*O*- β -D-GALACTOPYRANOSYL-D-GLUCOPYRANOSE)

removed minerals and residual protein from the permeate, but an obvious disadvantage is the need for constant regeneration of these ion exchange resins with acid or base. This problem can be eliminated by using types of ion exchange resins that are regenerated with hot water (Parrish *et al.* 1979A). This process is less expensive, nonpolluting, and yields lactose of analytical purity. Deionization of whey permeates by these methods results in pure lactose solution that must still be evaporated—an energy intensive step. Methods have been developed to recover lactose from dilute solutions by low energy methods, including precipitation with $\text{Ca}(\text{OH})_2 + \text{NH}_4\text{Cl}$ (Olano *et al.* 1977A) or $\text{CaCl}_2 + \text{NaOH}$ (Olano *et al.* 1977B). Using these methods in model systems, nearly 90% of lactose could be precipitated from dilute (5%) lactose solutions, especially when organic solvents (acetone and alcohols) were added to the solution (Madj and Nickerson 1976; Olano *et al.* 1977B; Nickerson 1979). The precipitation has been reported to result from the formation of an insoluble calcium-lactose complex (McCommins *et al.* 1980).

Properties and Uses of Lactose

Chemical and Physical Properties. Crystalline Forms. The most commonly prepared form of lactose is the alpha monohydrate (α -lactose·H₂O), which readily crystallizes from supersaturated lactose solutions at any temperature below 93.5°C (Nickerson 1974). Two less common and less easily prepared forms are the anhydrous alpha and beta anomeric forms. Various anhydrous alpha forms such as stable anhydrous α -lactose (α_s) are known. These alpha and beta forms differ in physical and chemical properties. The low initial solubility of α -lactose·H₂O precludes its use in many "instant" food products. β -Lactose, on the other hand, is initially more soluble and sweeter than α -lactose·H₂O. α_s is nonhygroscopic and more easily stored than other forms. Because of the useful properties of these less common forms, several recent methods have been developed for their preparation.

Anhydrous Forms of α -Lactose. Two forms of anhydrous α -lactose can be prepared by heating α -lactose·H₂O (Nickerson 1974). An unstable hygroscopic anhydrous α -lactose (designated α_H) is produced by heating α -lactose·H₂O at 130°C *in vacuo*. A stable anhydrous form of α -lactose is prepared by heating α -lactose·H₂O at 130°C in air. Lim and Nickerson (1973) prepared another stable anhydrous form of α -lactose by refluxing α -lactose·H₂O in methanol. Other alcohols, such as ethanol, *n*-propanol, *n*-butanol, and isobutanol, have also been effec-

tively used for this process (Nickerson and Lim 1974). Originally, it was assumed that the stable anhydrous α -lactose formed under these alcoholic treatments was identical to the α_s produced by heating α -lactose·H₂O in air. Ross (1978) however, using differential scanning calorimetry, compared α_s with the stable anhydrous α -lactose formed by methanol treatment of α -lactose·H₂O (designated α_m) and found the two to differ in melting point, heat capacity, and heat of fusion. Parrish *et al.* (1979B) showed that the anhydrous α -lactoses prepared by treatment of α -lactose·H₂O with methanol (α_m), ethanol (α_e), *n*-propanol (α_p), *n*-butanol (α_b) were all distinct species (α_{ROH})—each contained small but measurable amounts of alcohol. This contamination can be significant if this process is to be used to produce a food grade stable α -lactose.

β -Lactose. Several methods have been developed for preparation of the sweeter and more soluble form of lactose, the beta anomer. Olano and Rios (1978) converted α -lactose·H₂O into β -lactose in nearly quantitative yield by refluxing the alpha form in methanol containing small amounts of sodium hydroxide. The reaction also was effective when ethanol, *n*-propanol, or *n*-butanol was used as the solvent (Olano 1977). Parrish *et al.* (1979D) converted α -lactose·H₂O into β -lactose in a similar fashion using potassium methoxide or potassium hydroxide as base. The anhydrous forms of α -lactose (α_{ROH} , α_s) could also be converted into β -lactose (Parrish *et al.* 1980A) if small amounts of β -lactose were included in the reaction. A summary of the effect of neutral and alkaline methanol treatments on the various forms of lactose are summarized in Fig. 7.2.

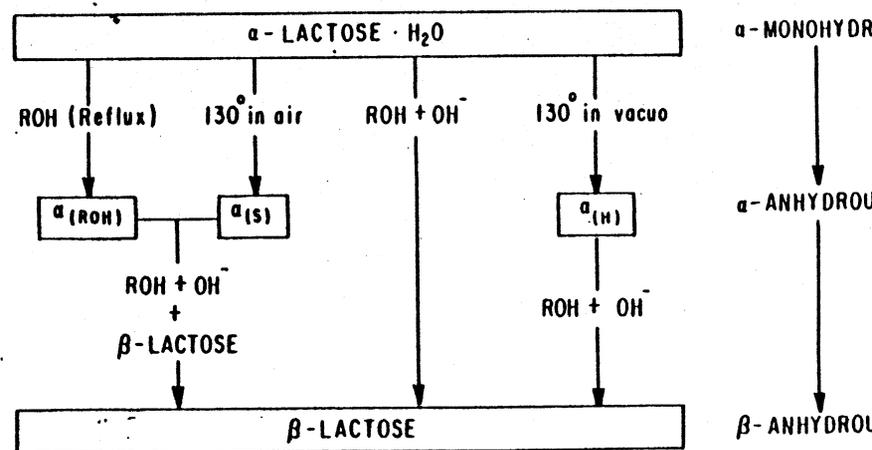


FIG. 7.2. CONVERSION OF α -LACTOSE·H₂O INTO ANHYDROUS α -AND β -LACTOSE

Biochemical and Physiological Properties. *Talor.* Because relative sweetness of sugars is concentration dependent, lactose can be anywhere from 15–30% as sweet as sucrose (Nickerson 1974). β -Lactose is significantly sweeter than α -lactose (Nickerson 1974; Parrish 1981), but since solutions of either anomeric form mutarotate to the same equilibrium composition of α - and β -anomers, there is no advantage in using either form except in the case of “instant”-type quick-dissolving applications.

Nutritional Significance. The reasons for lactose being the carbohydrate source for developing mammals is unclear, but several possibilities have been discussed (Hansen and Gitzelmann 1975). It is interesting that lactose is not always an acceptable carbohydrate source for adult mammals, particularly humans. It is estimated that nearly 70% of the world adult population is lactose intolerant (Paige *et al.* 1975). This is due to insufficient levels of the intestinal β -galactosidase (lactase) necessary for hydrolysis of lactose into its readily absorbable component monosaccharides, glucose and galactose. When lactose is not hydrolyzed and absorbed from the small intestine, it enters the colon, usually resulting in osmotic diarrhea, intestinal cramps, and microbially mediated gas and acid production.

Current Food Uses for Lactose. Several of the current food uses of lactose have recently been reviewed by Thelwall (1980). These include increasing the solids content of dairy products, fortifying infant formulas, and partially replacing sucrose in bakery products. Recent research (Nickerson 1979; Marvin *et al.* 1979; Parrish *et al.* 1979B) indicates that lactose may be an ideal adsorbant and carrier for natural and synthetic flavors, as several forms of the sugar efficiently adsorb and slowly release alcohols, esters, ketones, and aromatic hydrocarbons. However, due to the low solubility and low sweetness of lactose and widespread lactose intolerance, its food uses are always limited. For these reasons current research has been directed toward the conversion of lactose into more useful potential food carbohydrates.

Reactions of Lactose to Yield Potential Food Carbohydrates

Several enzymatic and chemical reactions of lactose lead to potential food carbohydrates. The reactions covered in this review (Fig. 7.3) will be reduction, hydrolysis, and isomerization, which yield lactitol, glucose and galactose, and lactulose, respectively.

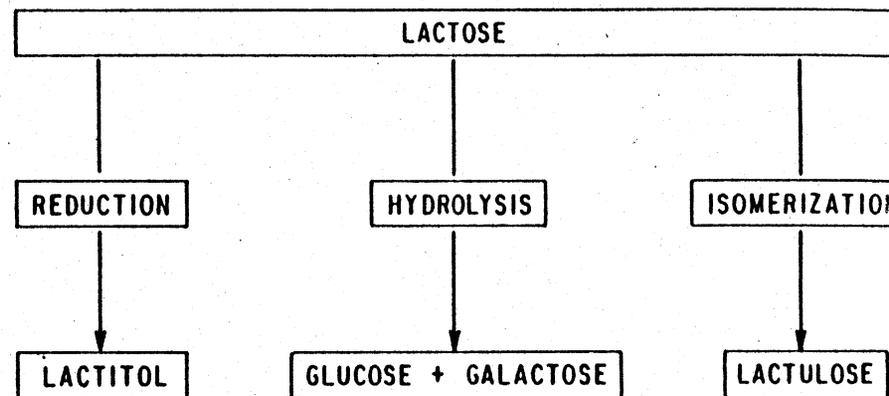


FIG. 7.3. REACTIONS OF LACTOSE THAT YIELD POTENTIAL FOOD CARBOHYDRATES

Reduction to Lactitol. *Methods.* Lactitol (Fig. 7.4) is a disaccharide sugar alcohol prepared by chemical reduction of the glucose residue of lactose to a sorbitol group. Two recent papers (van Velthuisen 1979; Saijonmaa *et al.* 1978) and one review (Linko *et al.*) describe methods for this reduction. Hydrogenation of lactose with sodium amalgam or calcium amalgam catalysts, and reduction with sodium borohydride have been successfully performed. Hydrogenation with Raney nickel catalyst, however, is the most commonly used method today. Hydrogenation at 100°C for 6 hr and 8825 kPa gives lactitol in nearly quantitative yields.

Chemical and Physical Properties. Lactitol may be crystallized as a monohydrate with a melting range of 94°–97°C. This monohydrate is extremely soluble. At 25°C, 206 g will dissolve in 100 g water. At 75°C, 915 g will dissolve in 100 g of water (van Velthuisen 1979). Because lactitol is not a reducing sugar, it will not mutarotate in solution to less soluble or less desirable forms. The absence of a potential carbonyl group results in exceptional stability toward acid, base, heat, and non-enzymatic browning reactions. van Velthuisen (1979) reported that heating lactitol for 1 hr at 100°C at pH 13 causes no discoloration.

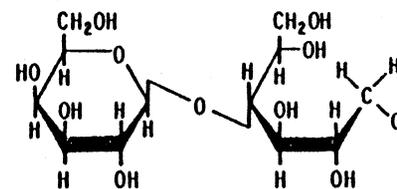


FIG. 7.4. STRUCTURAL FORMULA OF LACTITOL (4-O- β -D-GALACTOPYRANOSYL-D-SORBITOL)

whereas lactose under these conditions showed strong discoloration and degradation. Reports of the hygroscopic properties of crystalline lactitol monohydrate have been contradictory (van Velthuisen 1979; Saijonna *et al.* 1978) but it appears to be less hygroscopic than sorbitol, and more hygroscopic than mannitol. Lactitol solutions were reported to be much less hygroscopic than those of glycerol, sorbitol, or xylitol. Other physical properties of lactitol (solution densities and viscosities) have been reported by these authors.

Taste. Lactitol is reported to have a mild pleasant taste. It is slightly sweeter than lactose and about 30–35% as sweet as sucrose, depending on concentration.

Cariogenicity. Linko *et al.* (1980) reviewed early studies that showed lactitol was not readily fermented by oral bacteria. van Velthuisen (1979) reviewed *in vivo* studies of reduction of pH in dental plaque after consumption of foods containing lactitol or other sugars. Lactitol had a minor effect on tooth pH and was said to be "tooth-protective."

Bioavailability. Knowledge of the ability of human intestinal enzymes to hydrolyze and absorb lactitol is incomplete. In a patent (Hayashibara and Sugimoto 1976), it was claimed that lactitol is noncaloric because it is neither digested nor absorbed from the intestines of rabbits. van Velthuisen (1979) reviewed studies on the caloric potential of this sugar alcohol. After adult males consumed 24 g lactitol, none of the sugar was detected in urine, blood or feces. Blood glucose rise was also minimal. Blood galactose was not reported. It was assumed that the sugar alcohol was not digested, but fermented in the large intestine. More research is needed to determine the actual caloric significance of dietary lactitol.

Toxicity and Side Effects. van Velthuisen (1979) reviewed toxicity data on lactitol. The maximum level causing no toxicity in rats was 2.5 g/kg of body wt per day. Consumption of 5% dietary lactitol by rats led to diarrhea, but less than that caused by 5% xylitol or sorbitol. Humans reportedly adapt to regular consumption of lactitol and can consume up to 20 g in a single dose without unpleasant side effects.

Potential Food Uses. Lactitol is not currently approved by the FDA as a food additive, but its potential uses are numerous. Because of its limited sweetness and high solubility, it would function well as a bulking agent in various food products. The excellent chemical stability would allow its use in foods that undergo severe processing or storage conditions. Because of its possible noncaloric nature, lactitol may find

use in dietetic products. The maximum amounts allowed in foods would have to be regulated, due to possible laxative effects.

Hydrolysis of Lactose. Methods. The products from the hydrolysis of lactose are D-glucose and D-galactose (Fig. 7.5), two common monosaccharides. Two reviews describe many of the recent developments in lactose hydrolysis (Harju and Kreula 1980; Anon. 1979A). Processes for lactose hydrolysis can be divided into two groups, those that require acid catalyzed hydrolysis and those that utilize enzymatic methods.

Acid Catalyzed Hydrolysis. Vujicic *et al.* (1977) and Lin and Nielsen (1977) effectively hydrolyzed pure lactose solutions (5–40% w/wt) with 1–3 N HCl or H₂SO₄. By using relatively low temperatures (60°C) and long reaction times (up to 36 hr), they were able to hydrolyze 90% of the lactose into monosaccharides. This process was not adaptable to hydrolysis of lactose in concentrated whey because residual proteins and salts led to degradative side reactions, discoloration, and off-flavors that were not easily removed. Guy and Edmondson (1978) efficiently hydrolyzed lactose at a higher temperature (121°C), using 0.1 N HCl and short reaction times.

Strong acid (sulfonic acid-type) ion-exchange resins have also been used to catalyze lactose hydrolysis. The advantages of this catalyst are ease of purification of the hydrolyzate (no mineral acid to be removed), short reaction times, and continuous operation. Recently, MacBean *et al.* (1979) studied the hydrolysis of lactose in whey permeate and pulp solution by a number of commercially available cation-exchange resins. Excellent hydrolysis was achieved with strong acid, gelular-type cation exchange resins, with low degrees of crosslinking.

Enzymatic Hydrolysis. Lactose can be hydrolyzed by β-galactosidase (lactase) enzymes and the literature on hydrolysis with this class of enzymes is voluminous. Recent reviews (Harju and Kreula 1980; Anon. 1979A; Shukla 1975) discuss specific aspects of this method. Single batch hydrolysis processes have been developed, but are generally too expensive for commercial use. Continuous processes have now been developed in which the β-galactosidase is covalently immobilized on

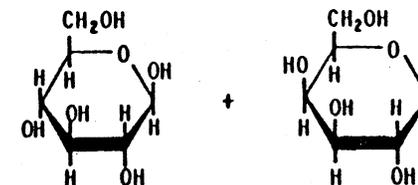


FIG. 7.5. PRODUCTS OF LACTOSE HYDROLYSIS: D-GLUCOSE AND D-GALACTOSE

solid support. Like continuous hydrolysis on cation-exchange resins, this process requires no post-removal of added reagents, and the stability of these immobilized enzyme columns has been impressive. One process developed by Corning Glass² (Moore 1980) involves a lactase bonded to porous, high surface area silica beads. The process is entirely automatic and achieves 80% hydrolysis of the lactose in demineralized whey ultrafiltrates. The percent hydrolysis remained constant for 100 production runs, each 16–20 hr in duration, and a 1- to 5-year system life was estimated.

Chemical and Physical Properties. Guy and Edmondson (1978) studied the solubility of hydrolyzed lactose syrups. Hydrolysis of lactose to the correct degree can lead to a large increase in solubility. Syrups with 75% hydrolysis and 63–66% solids showed maximal resistance toward crystal formation and microbial growth. Increasing the hydrolysis percentage above 75, however, supersaturates the solution with respect to galactose, which subsequently crystallizes. Below 75% hydrolysis, lactose is not totally soluble and will eventually crystallize from solution.

Taste. The sweetness of hydrolyzed lactose syrups varies with both the degree of hydrolysis and the sugar concentration (Guy and Edmondson 1978). Above 50% solids, hydrolyzed lactose syrups (75% hydrolyzed) are as sweet as sucrose syrups. Increasing lactose hydrolysis from 75 to 95% only slightly increases sweetness, so complete hydrolysis is not necessary. In addition, the three sugars present in these syrups, galactose, glucose and lactose, appear to behave synergistically. Their combined sweetness was higher than would be calculated from the sum of their individual sweetness values.

Bioavailability. Because much of the adult world population is unable to adequately digest lactose due to β -galactosidase deficiency, prehydrolysis of this disaccharide can increase bioavailability of the carbohydrate. Both glucose and galactose are actively transported from the small intestine. Research on the transport and the metabolism of these sugars has been reviewed (Hansen and Gitzelmann 1975).

Toxicity and Side Effects. Lactose is a common ingredient in many human foods and is often added to processed food in the form of regular or modified whey solids. In the United States, lactase-treated milks and

dairy products are commercially available. The use of hydrolyzed lactose syrups as food additives, however, is not currently approved by the Food and Drug Administration. Early research indicated that high levels of free galactose in the diet led to high serum galactose levels and eventually to cataract formation in experimental animals (Kinoshita 1974). A recent review (Anon. 1979A) indicated that when galactose and glucose are fed together in equal concentrations, blood galactose was significantly lower than when galactose was fed alone. A consumption of 40 g of galactose produced a blood galactose of 0.61 g/liter, whereas 40 g galactose and 40 g glucose together gave a blood galactose of only 0.14 g/liter. When massive amounts of hydrolyzed lactose syrups were fed to baboons for ten weeks, no adverse physical or metabolic effects were noted. A study with lactose intolerant people in London revealed that hydrolyzed lactose was digested without side effects.

Potential Food Uses. Hydrolyzed lactose syrups have a variety of potential food applications. Besides being used as specialty sweetener for lactose intolerant people, their high sweetness and solubility would permit use as replacements for corn derived sweeteners and sucrose. This is especially important in Europe where corn derived sugars are not as readily available as in the United States (Anon. 1979B). Specific applications as reviewed by Harju and Kreula (1980) include uses in ice cream, caramels, bakery products, canned fruits, and wine and beer production.

Isomerization of Lactose to Lactulose. *Methods.* Lactulose (Fig. 7.6) was first prepared by Montgomery and Hudson (1930) by isomerization of lactose in saturated lime water. Isomerization of aldoses into ketoses is a well-known reaction in carbohydrate chemistry and is catalyzed by acid or base (Speck 1958). Preparation of ketoses by this method is tedious, as the yields are low (<20%) and the ketose must be isolated from unreacted starting material, alkaline degradation products, and metal salts. More recent methods for producing lactulose have involved the use of complexation reagents such as aluminate (Guth and Tummerman 1970) and borate (Mendicino 1960). These compound

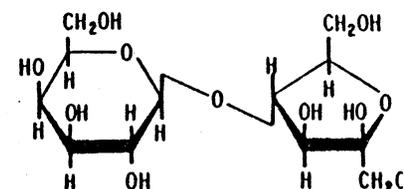


FIG. 7.6. STRUCTURAL FORMULA OF LACTULOSE (4-O- β -D-GALACTOPYRANOSYL-D-FRUCTOSE)

²Reference to a brand or firm name does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

shift the pseudoequilibrium established during base-catalyzed isomerization in favor of the ketose and prevent degradative side reactions. These reagents permit formation of lactulose in high yield, but both are impractical since a large excess of borate is necessary for optimum yield (Mendicino 1960), and aluminate is removed from the product only with great difficulty. Attempts to use immobilized borate (Carubelli 1966) or aluminate (Rendleman and Hodge 1979) on ion exchange resins as isomerization catalysts are promising from an analytical standpoint but are not practical for preparative purposes. Recently, a method was developed (Hicks and Parrish 1980) to prepare lactulose in nearly 90% yields by treatment of lactose with boric acid in an aqueous solution made basic by tertiary amines. The reaction rate is proportional to pH (Table 7.1) and temperature (Table 7.2). All tertiary amines tested (Table 7.3), were capable of catalyzing the reaction but not all were basic

TABLE 7.1. INFLUENCE OF pH ON REACTION YIELD

pH	Yield of:	
	Lactulose (%)	Monosaccharides (%)
9.0	6	<1
10.0	32	5
10.5	74	3
11.0	83	3

Source: Hicks and Parrish (1980).
¹ Reaction temperature was 40°C; Reaction time was 96 hr. Yield measured by quantitative HPLC.

TABLE 7.2. EFFECT OF TEMPERATURE ON YIELD OF LACTULOSE

pH	Temperature/time	Lactulose (%)	Monosaccharides (%)
11.0	40°C/96 hr	83	5 ¹
11.0	70°C/4 hr	87	3

Source: Hicks and Parrish (1980).

TABLE 7.3. EFFECT OF TERTIARY AMINE ON YIELD OF LACTULOSE

Amine	pH	Lactulose (%)	Monosaccharides (%)
1,4-Dimethylpiperazine	9.5	21	<1
N-methylpiperidine	10.8	81	<1
N,N,N',N'-tetramethylethylenediamine	9.7	33	5
Triethylamine	11.0	87	3

Source: Hicks and Parrish (1980).

and water soluble enough to titrate the reaction solution to the optimum level (pH 11) and, hence, required longer reaction times for maximum yields. Alkaline degradation reactions at pH 11 were minimal, as the solutions were colorless, high yields of lactulose were produced, and only low levels of monosaccharides were present. The combination of triethylamine and boric acid was uniquely useful in that they could be recycled for subsequent reactions (Fig. 7.7), adding to the efficiency of the process.

Chemical and Physical Properties. Lactulose is an extremely soluble sugar in water and polar organic solvents such as methanol. A saturated aqueous solution at 30°C contains about 77% w/w lactulose (Oosten 1967A). Unlike lactose, this ketose disaccharide does not crystallize from concentrated solutions after long storage. In fact, lactulose is a difficult sugar to crystallize, especially when trace quantities of other sugars are present. Difficulty in preparing pure crystalline lactulose led to conflicting reports about the structure in the solid state. Montgomery and Hudson (1930) crystallized lactulose from 50% methanol (mp 150°C) and suggested a 4-β-D-galactosido-α-D-fructose structure. Isbell and Pigman (1938) suggested, based on polarimetric data, that the fructose was in a furanose form. Oosten (1967B) crystallized

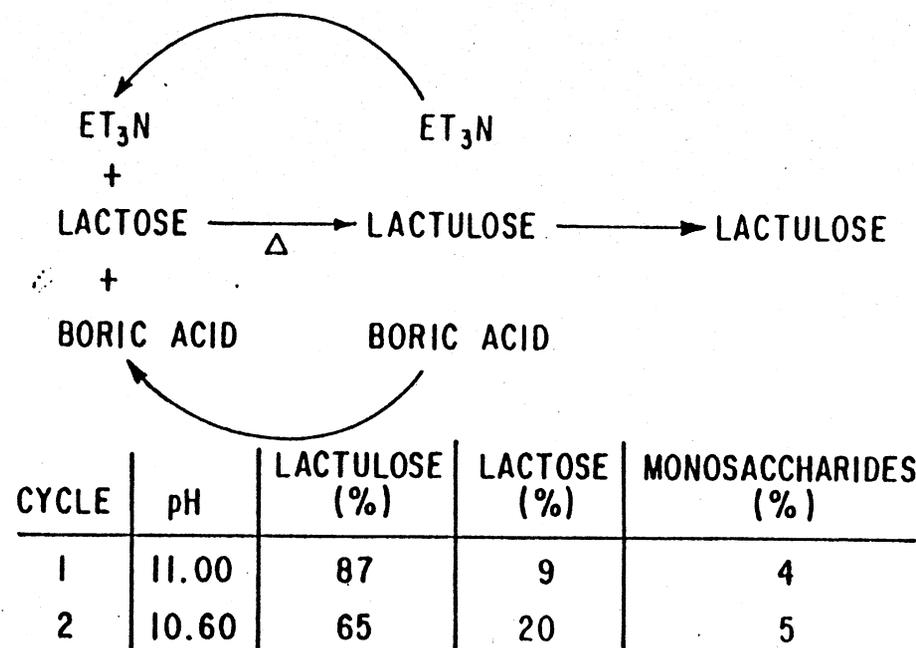


FIG. 7.7. RECYCLING OF REAGENTS FOR LACTULOSE SYNTHESIS

lactulose from boiling methanol. The snow-white, transparent crystals melted between 168.5°C and 169°C and were said to be in the "α-form." Perlin *et al.* (1973) examined the anomeric composition of lactulose (freshly dissolved in DMSO- d_6) by proton nuclear magnetic resonance spectroscopy. They concluded that this crystalline form was mostly the β-D-fructofuranose anomer with trace amounts of β-D-fructopyranose and α-fructofuranose forms. Pfeffer and Hicks (1980) examined lactulose that had been crystallized from refluxing methanol (mp 169°–171°C) by solid state, magic angle spinning cross polarization ^{13}C NMR spectroscopy. The reducing moiety in the crystalline solid consisted of a mixture of β-fructofuranose : β-fructopyranose : α-fructofuranose forms in a ratio of about 15 : 3 : 2. Despite varied crystallization methods, no anomerically pure crystalline form could be obtained.

By virtue of the ketose functionality and 4-*O*-glycosyl substitution, lactulose is extremely unstable in alkaline solution. The compound degrades by alkaline-peeling and β-elimination reactions to give galactose, isosaccharinic acids and other acidic products (Corbett and Kenner 1954). It is also susceptible to amine-assisted dehydration and degradation reactions (Hough *et al.* 1953). The humectant properties of lactulose were the subject of several studies. Preliminary studies on impure lactulose preparations (Ross *et al.* 1979) suggested lactulose was approximately twice as effective as sucrose in lowering solution water activity. Later studies (Chirife and Ferro-Fontan 1980; Huhtanen *et al.* 1980) showed lactulose was very similar to sucrose in humectant properties.

Taste. The sweetness of lactulose solutions was studied over a concentration range of 5–35% (w/w) (Parrish *et al.* 1979C). Under these conditions, the sweetness was 48–62% that of sucrose.

Cariogenicity. Lactulose caused the pH of a suspended salivary system to decrease much less than did sucrose (Mäkinen and Rekola 1975). The oral microorganisms produced more lactic acid in the system when grown on sucrose as compared to lactulose. Mayo (1981) concluded that oral microorganisms growing on lactulose produced acid at a slower rate than those growing on a sucrose solution. With microelectrodes implanted *in situ*, it was demonstrated that oral bacteria can ferment lactulose in dental plaque, but lactulose was significantly less acidogenic than sucrose.

Bioavailability. It is currently believed that lactulose is not metabolized by human alimentary enzymes. Dahlqvist and Gryboski (1965) were first to demonstrate the inability of human intestinal enzymes to hydrolyze lactulose into absorbable monosaccharides. Thus, even indi-

viduals who are lactose tolerant can not digest lactulose. Presumably lactulose is fermented in the large intestine to a mixture of organic and other unknown compounds.

Toxicology and Side Effects. Few studies have reported toxicological data on lactulose, but it is consumed in large quantities (up to 30 g/day) by individuals undergoing specific medical treatments. Flatulence and diarrhea can appear in these cases, but these effects usually be avoided by carefully reducing the intake. No other effects have been noted (Mendez and Olano 1979).

Uses. Lactulose has several important uses in the food and pharmaceutical industry. It is claimed that the presence of lactulose in infant formula encourages the development of *Bifidobacterium bifidum* in the intestinal flora, mimicking the flora found in healthy breast-fed infants (Mendez and Olano 1979). The two major uses for the sugar are in the treatment of portal systemic encephalopathy and chronic constipation. Portal systemic encephalopathy is a hepatic coma condition which occurs in individuals with impaired, cirrhotic livers. The cause is largely unknown, but is believed to result from high levels of nitrogen-containing compounds in the blood. It has been suggested that the colonic acidosis produced by lactulose lowers the level of these toxic compounds, but further research is needed to completely understand the mechanism involved (Conn 1978). When taken in large doses, lactulose has been shown to be an effective laxative, especially for those suffering from chronic constipation. Lactulose is an advantageous laxative in these cases because the body does not develop an increasing tolerance to the drug as in the case of many other common laxatives (Anon. 1979). It has been suggested (Parrish *et al.* 1979C) that lactulose could be a partial replacement for sucrose and corn sweeteners in intermediate moisture foods. The high solubility, intermediate sweetness, and low caloric qualities of this unique disaccharide would appear to make it an ideal candidate for food use. Because of its laxative properties, however, only limited amounts could be tolerated in most food products. It is currently approved for food use by the FDA.

Analytical Methods for Lactose and Its Derivatives

An excellent review (Harper 1979) recently appeared describing various methods for the analysis of lactose and its derivatives. This review will only briefly outline those methods and comment on their general usefulness for specific application. Recent developments in high performance liquid chromatography will be covered in more detail.

Methods for the quantitative analysis of lactose and its derivatives can be grouped into the categories in Table 7.4. Polarimetry is extremely useful for determining the anomeric form of crystalline lactose or related compounds. Quantitation of lactose by polarimetric analysis is limited, however, to samples that are free of other unknown optically active compounds. Colorimetric methods are often used for the analysis of these carbohydrates. Samples that contain one major sugar can be easily analyzed by these techniques, but samples with three or four sugars may require several different colorimetric analyses to accurately determine the composition (Parrish *et al.* 1980B). Enzymatic methods are generally accurate and extremely specific, but, as in the case of colorimetric analyses, some complex samples may require multiple analysis.

Several simple but accurate cryoscopic methods have been developed to measure the percentage hydrolysis of lactose in whey and lactose solutions (Baer *et al.* 1980) and the amount of lactose in milk products (Zarb and Hourigan 1979). Gas-liquid chromatographic (GLC) (Harper 1979) and high performance liquid chromatographic (HPLC) methods have been developed for analysis of these sugars. Both methods offer advantages over the previously mentioned techniques, in that several sugars can be quantitated in the same chromatographic analysis. Sugars must be converted into volatile derivatives before analysis by GLC, adding to analysis time and complexity. Sugars may be directly analyzed by HPLC without formation of derivatives and usually without extensive sample cleanup. HPLC methods have been developed to quantitate lactose in fluid milk, ice cream, yogurt, dry milk (Hurst *et al.* 1979; Warthesen and Kramer 1979; Euber and Brunner 1979) and chocolates (DeVries *et al.*; Hurst and Martin 1980). These methods require commercially available columns pre-packed with high-performance silica particles that have been modified with a polar amino or cyano-type bonded phase. Mobile phases in the systems consist of simple acetonitrile/water mixtures and when flow rates of 1–2 ml/min are used, the retention time for lactose is generally 10–20 min. Recently, Parrish *et al.* (1980B) developed an HPLC method for the analysis of lactulose syrups. The four sugars present, lactose, lactulose, galactose and tagatose were all

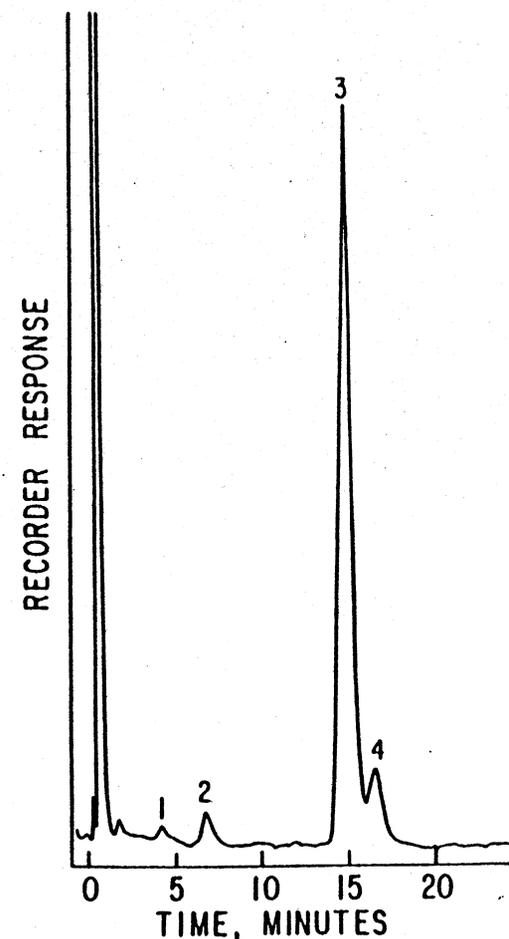
TABLE 7.4. MAJOR METHODS FOR QUANTITATION OF LACTOSE AND ITS DERIVATIVES

Polarimetry
Colorimetry
Enzymatic
Cryoscopy
Gas-liquid chromatography
High-performance liquid chromatography

separated on a Waters μ bondapak/carbohydrate column (Fig. 7.8). The column was eluted at 2 ml/min with a mobile phase consisting of 77% (wt/wt) acetonitrile/water. Sugars were detected with a differential refractometer. The retention times, capacity factors, and relative detector responses for the four sugars are listed in Table 7.5. Sugars can be conveniently quantitated by this method, by comparison of peak heights with those of corresponding sugars in standard solutions. A commercial lactose syrup, Cephulac, was analyzed by this technique, and its sugar composition is shown in Table 7.6.

Summary and Future Research Needs

A summary of the properties of lactose and its derivatives is given in Tables 7.7 and 7.8. If, and when, FDA approval is granted for the use



From Parrish *et al.* (1980)
 FIG. 7.8 HIGH PERFORMANCE LIQUID CHROMATOGRAM OF A LACTULOSE SYRUP. Components are (1) tagatose; (2) galactose; (3) lactulose; and (4) lactose.

TABLE 7.5. LIQUID-CHROMATOGRAPHIC EVALUATION OF STANDARD SUGARS

Sugar	Retention time, t_r (min)	Capacity factor k'	Relative response
Tagatose	4.50	1.40	3.88
Galactose	6.72	2.54	1.38
Lactulose	13.40	6.07	1.53
Lactose	15.50	7.30	1.00

Source: Parrish *et al.* (1980).

TABLE 7.6. ANALYSIS OF SUGARS IN CEPHULAC¹

Sugar	Trial					Statistic		
	1	2	3	4	5	\bar{x}	SD	CV (%)
Tagatose	1.12	0.98	1.08	1.10	0.94	1.04	0.08	7.60
Galactose	12.58	12.32	12.35	12.63	12.31	12.44	0.15	1.24
Lactulose	79.45	80.03	79.95	79.10	80.03	79.71	0.42	0.52
Lactose	6.86	6.66	6.61	7.17	6.72	6.80	0.22	3.31

Source: Parrish *et al.* (1980).

¹ In relative weight percent.

TABLE 7.7. PHYSICAL AND CHEMICAL PROPERTIES OF LACTOSE AND LACTOSE DERIVATIVES

Compound	Water solubility	Chemical stability
Lactose	Low	Moderately stable in weak acid. Unstable in base.
Lactitol	High	Moderately stable in weak acid. Stable to thermal and base catalyzed degradative processes.
Lactulose	High	Moderately stable in weak acid. Very unstable in base.
Lactose hydrolysates	Moderately high	Stable in weak acid. Unstable in basic solution.

TABLE 7.8. PHYSIOLOGICAL EFFECTS OF LACTOSE AND LACTOSE DERIVATIVES

Compound	Physiological Effects		
	Sweetness	Nutritive value	Side effects
Lactose	Low	Completely metabolized	Few
Lactitol	Low	Probably not metabolized	Laxative
Lactulose	Moderate	Not metabolized	Laxative
Lactose hydrolysates	Moderate to high	Completely metabolized	Few

these derivatives in foods, they will find many applications. More search is needed to determine the nutritional safety and significance of these potential food carbohydrates.

HONEY

Introduction

Honey is the only sweetener that can be stored and used as it naturally produced and is essentially a highly concentrated aqueous solution of the two sugars, glucose and fructose. Mention of honey can be found throughout recorded history. It was man's first sweetener and remained so until being largely supplanted over the past 100 years by cane, beet, and corn-derived sugars. Research has resulted in the identification of many components in honey other than glucose and fructose, including higher sugars, enzymes, organic acids, and minerals. Honey is quite variable in most respects, but particularly with regard to color, flavor, moisture, and sugar content. These characteristics depend primarily on floral source, climate, and beekeeping practices. Volun have been written describing beekeeping practices and the processes by which the honeybee converts the relatively simple raw materials, nectar and honeydew, into honey. This review will emphasize the sugars in honey; how they have been identified, quantitated, and produced by the honeybee.

The Precursors—Nectar and Honeydew

There are two general types of honey: floral and honeydew. The sugars in floral honey originate in the nectar of various flowers. Hundreds of different plants provide the honeybees with nectar, and while it contains traces of minerals, organic acids, amino acids, vitamins, pigments, proteins, and aromatic compounds, the solids of nectar consist mainly of the sugars sucrose, fructose, and glucose. A wide natural variation in both the ratio of these sugars and in their total concentration (normally 20–40%) occurs in nectar. There have been reports of small quantities of maltose, raffinose, and melezitose being found in nectar. Honeydew is obtained by the honeybee indirectly, from sweet syrups excreted by various hemipterous insects feeding on tree phloem sap. The essential difference between the composition of nectar and honeydew is that the latter usually contains significant quantities of the trisaccharides melezitose and erlose. These are produced from sucrose in plant phloem by plant-sucking insects. A detailed discussion of nectar and honeydew appears elsewhere (Maurizio 1975).

The Nectar to Honey Transformation. It was long thought that honey was little more than an inverted and concentrated nectar syrup, consisting only of the sugars fructose, glucose, sucrose, maltose, and an ill-defined material termed "honey dextrin." Now it is apparent that many complex physical and chemical processes occur during the conversion of nectar and honeydew to honey. *Ripening* is the term applied to the sum of all these processes.

The primary physical change that occurs during the nectar to honey conversion involves reducing the water content of nectar to less than 20%. Foraging honeybees return to the hive and pass their load (about 50 mg) of material to the house bees. These bees alternately expel and ingest small droplets of the nectar with their mouthparts while collectively fanning their wings to increase air movement and evaporation. This reduces moisture from about 80 to 40%. The rest of this moisture is removed after placing the half-ripened honey in the comb cells—again by rapidly moving the air in the hive. When the moisture level is below 20%, the cells are capped with wax. Some consequences of this low water level, such as crystallization and unsuitability for microbial growth and fermentation, will be discussed later.

A number of chemical changes occur during and after the water removal period. These changes affect the nectar sugars and result primarily from the hypopharyngeal gland secretions by the honeybee into nectar. The primary change is the inversion of nectar sucrose by honeybee invertase. The nectar itself may, in some cases, contain invertase, and its different catalytic properties may explain why some honeys are much higher in fructose than most (Robinia and Tupelo), and why others are higher in glucose (dandelion, rapeseed and blue curls). The role of other enzymes, including amylases, glucose oxidase, and catalase in the ripening process, will be described later. Overall, the honey-making process is very efficient. To produce 454 g of honey, a bee colony uses approximately one-fifth of the honey to maintain the colony, and the bees fly some 75,000 miles. A comprehensive review of the nectar to honey transformation has been published (Maurizio 1975).

Honey—Gross Composition

The variability in composition of both floral and honeydew honey is shown in Table 7.9, which summarizes the results of the most comprehensive survey (504 samples) of United States honeys (White *et al.* 1962). Honey is above all a very complex carbohydrate material, with 95–99.9% of the solids being sugars. Fructose and glucose are the major sugars, accounting for about 85% of these solids. It is very important for the moisture level in honey to be close to the mean value of 17.2%, so

that it will not granulate and ferment. Standards of the U.S. Department of Agriculture require that less than 18.6% water be present for grading, and retail honey is usually blended to 18.0% or less water.

Many sugars, some quite rare, have been identified in honey over the past 30 years. So the early ill-defined "honey dextrin" fraction is in fact an exceedingly complex mixture that includes not only sucrose and maltose, but also eight other disaccharides, ten trisaccharides, and two higher saccharides. All of these sugars consist only of glucose and fructose, linked in various fashions. The material listed as maltose in Table 7.9 actually includes all other reducing disaccharides in honey.

TABLE 7.9. COMPOSITION OF UNITED STATES FLORAL (490 SAMPLES) AND HONEYDEW (14 SAMPLES) HONEY

Composition	Average (%)	Range (%)	S.D. (%)
Floral honey			
Moisture	17.20	13.4 – 22.9	1.46
Fructose	38.19	22.25–44.26	2.07
Glucose	31.28	22.03–40.75	3.03
Sucrose	1.31	0.25– 7.57	0.95
"Maltose"	7.31	2.74–15.98	2.09
Higher sugars	1.50	0.13– 8.49	1.03
Undetermined	3.10	0.0 –13.2	1.97
Honeydew honey			
Moisture	16.30	12.2 –18.2	1.74
Fructose	31.80	23.91–38.12	4.16
Glucose	26.08	19.23–31.86	3.04
Sucrose	0.80	0.44– 1.14	0.22
"Maltose"	8.80	5.11–12.48	2.51
Higher sugars	4.70	1.28–11.50	1.01
Undetermined	10.10	2.70–22.4	4.91

Source: White *et al.* (1962).

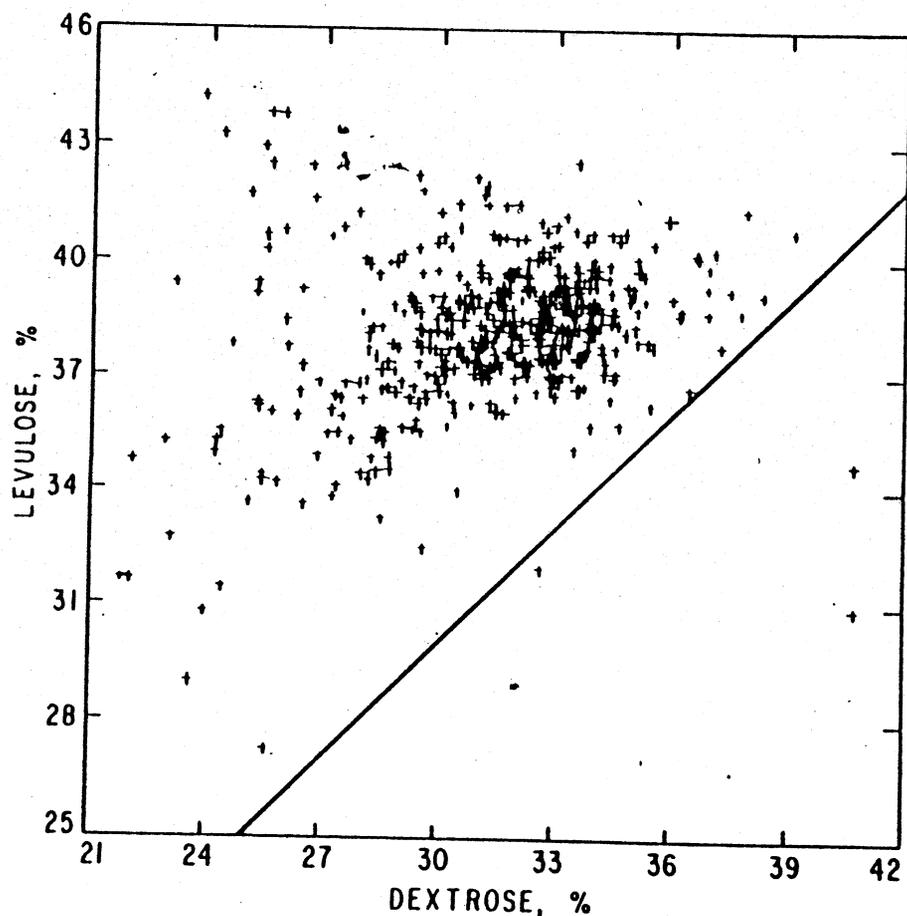
Small quantities of proteins, amino acids, organic acids, vitamins, minerals, and plant derived organic compounds are present in honey. These are responsible for the variations in color, flavor, and aroma among honeys. The protein fraction includes of course, the enzymes which are added by the honeybee for ripening. Honey is a dynamic system, continuing to undergo changes well after harvesting and throughout storage. The mineral content of honey, as reflected in the ash content, is low and quite variable, and potassium predominates. With the vitamins, amino acids, and proteins, their level is too low to be of any nutritional significance. The composition of honey has been described in detail (White 1978) and further references are found there.

Fructose and Glucose—The Honey Monosaccharides

Fructose is, with few exceptions, the predominant sugar in honey, averaging 38.19%. Glucose is normally present in lesser amounts, av

aging 31.28%. These two monosaccharides account for a much greater percentage of the total solids in honey than they do in nectar and arise from the inversion of nectar sucrose by honeybee invertase. Fig. 7.9 shows the values for the levulose (fructose) : dextrose (glucose) ratio found in the comprehensive survey of 457 United States honey samples (White *et al.* 1962). Only three of the samples had a ratio of less than 1.0. The primary determinant of this ratio is floral source; geographical origin and seasonal influences are minor.

All surveys of honey composition prior to 1950 were conducted under the assumption that the only reducing sugars present were glucose and



From White (1978)

FIG. 7.9. LEVULOSE (FRUCTOSE) : DEXTROSE (GLUCOSE) RATIOS IN 457 HONEY SAMPLES.
Line represents ratio of 1.00.

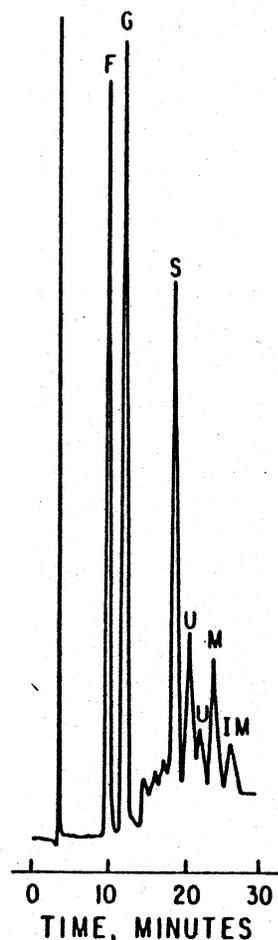
fructose. The methods used, reducing sugar and polarimetric analysis did not truly reflect the monosaccharide levels in honey, since there were several higher sugars present, with different optical rotations and degrees of reducing power. In these early surveys, variance due to method were as great as that due to differences among various types of floral honey.

The new categories of honey sugars (White and Maher 1954), including reducing di-, tri-, and higher saccharides, were discovered by employing a new method which fractionates the honey sugars according to size. This method, termed the selective adsorption procedure, utilized charcoal-celite column chromatography (Whistler and Durso 1950). It allowed the fractionation of honey into monosaccharides, disaccharides, and higher saccharides before analysis. In the selective adsorption procedure, the honey sugars are separated into classes by eluting them from charcoal-celite columns with aqueous solutions of increasing ethanol content. Monosaccharides are eluted with 1% ethanol, disaccharides with 7%, and the higher oligosaccharides with 50% aqueous ethanol.

The selective adsorption procedure was used in the 1962 survey (White *et al.* 1962), so that fructose and glucose could be determined without interference from other sugars. Glucose was determined by hypiodite oxidation and fructose then determined by copper reduction. A correction was included for hypiodite oxidation of fructose. The selective adsorption procedure can be quite time-consuming if many analyses are required. High performance liquid chromatographic (HPLC) (Thean and Funderburk 1977) and gas liquid chromatographic (GLC) (Wood *et al.* 1975; Doner *et al.* 1979) methods have been applied to honey sugar analysis. GLC appears to be adequate for the determination of fructose, glucose, and some of the higher sugars, but requires conversion of these sugars to volatile derivatives. HPLC has been used to great advantage, and the monosaccharides can be precisely determined without the need for preliminary class fractionation. Glucose, fructose, and sucrose are baseline resolved, using normal phase HPLC on amino propyl silica columns. Peaks are quantified by comparing the response to refractive index detection of the honey sugars with responses of a standard mixture of sugars. A typical chromatogram of honey is shown in Fig. 7.10.

The most important enzyme in honey, invertase, is responsible for converting nectar sucrose into fructose and glucose. Since the discovery of invertase in honey, much effort has been devoted to ascertaining whether honey invertase originates in the honeybee or in the nectar pollen. It is now accepted that most is added by the honeybee during

FIG. 7.10. HIGH PERFORMANCE LIQUID CHROMATOGRAM OF THE HONEY SUGARS. (F) Fructose; (G) Glucose; (S) Sucrose; (M) Maltose; (IM) Isomaltose; (U) unidentified. Refractive index detection with attenuation 32X for FG; 2X for S,M,IM,U.



collection of nectar and the ripening process. It has not been established that any plant invertase is present in honey.

Honeybee invertase continues its activity in extracted honey unless destroyed by heating. This invertase is an α -glucosidase, and possesses transglucosylase activity, in contrast with yeast invertase, which possesses transfructosylase activity. Being a transglucosylase, honeybee invertase, in addition to hydrolyzing sucrose, can transfer α -glucosyl units from sucrose to suitable acceptors. In its reaction with sucrose, six oligosaccharides are formed, which are all eventually hydrolyzed to glucose and fructose. The major intermediate is erlose (α -maltosyl- β -D-fructofuranoside), formed by the transfer of a glucose moiety from sucrose to the 4-hydroxyl of glucose in an intact sucrose molecule. In concentrated sucrose solutions, erlose can accumulate to levels of 11%.

The additional oligosaccharides from sucrose-invertase interaction produced by stepwise addition of α -D-glucosyl units to the 4-hydroxy previously added glucose units.

The transference of glucose to acceptors accounts, in part, for the ratio of fructose to glucose in honey being greater than one. Another enzyme is added to nectar by the honeybee which also effects the fructose : glucose ratio. This is glucose oxidase, which produces gluconic acid and hydrogen peroxide from glucose. Gluconic acid is the principal acid in honey, and the production of this acid and hydrogen peroxide is thought to assist in preserving nectar from spoilage during ripening. A comprehensive discussion of the honey enzymes appears elsewhere (Whitney 1978).

The Honey Di-, Tri-, and Higher Saccharides

Detailed reviews describing the identification of the higher sugars in honey have been published elsewhere (Siddiqui 1970; Doner 1977); they will be summarized here. It can be seen in Table 7.9 that there is wide variability in the total levels of the reducing disaccharides (list as maltose) and higher sugars in honey. The sugars which have been unequivocally identified in honey, are listed in Table 7.10, along with the three research groups responsible for their identification. In addition to glucose and fructose, ten disaccharides, ten trisaccharides, and two higher sugars are present, including most of the known sugars which contain glucose and/or fructose. It was necessary to isolate and characterize each, a considerable task when one considers the minute levels at which most are present. Sucrose and maltose were identified early, and all others since 1959. Each of the three groups used charcoal-celite chromatography to fractionate the honey sugars according to size. Isomaltose, maltulose, nigerose, and turanose were identified in the disaccharide fraction after further separations by preparative paper chromatography and stearic acid-treated charcoal column chromatography. This latter column method had earlier been shown to resolve some disaccharides not separable by paper chromatography. The separated disaccharides were converted to their β -octaacetates and identified by comparing their infrared spectra with those of standards. Confirmation was achieved by paper electrophoresis of the free sugars with standards. Kojibiose was characterized as its crystalline octaacetate after isolating it from honey by gradient elution from a charcoal-celite column, followed by re-chromatography on a charcoal-celite column with pH 10 borate buffer. The remaining di- and trisaccharides were resolved by various methods after first obtaining, by charcoal-celite chromatography, 67 g of the oligosaccharide fraction from 2 kg of honey.

TABLE 7.10. THE SUGARS OF HONEY

Trivial name	Systematic name
Glucose	
Fructose	
Sucrose	α -D-glucopyranosyl- β -D-fructofuranoside
Maltose	O - α -D-glucopyranosyl-(1 \rightarrow 4)-D-glucopyranose
Isomaltose	O - α -D-glucopyranosyl-(1 \rightarrow 6)-D-glucopyranose ¹
Maltulose	O - α -D-glucopyranosyl-(1 \rightarrow 4)-D-fructose ¹
Nigerose	O - α -D-glucopyranosyl-(1 \rightarrow 3)-D-glucopyranose ¹
Turanose	O - α -D-glucopyranosyl-(1 \rightarrow 3)-D-fructose ¹
Kojibiose	O - α -D-glucopyranosyl-(1 \rightarrow 2)-D-glucopyranose ²
Laminaribiose	O - β -D-glucopyranosyl-(1 \rightarrow 3)-D-glucopyranose ³
α , β -Trehalose	α -D-glucopyranosyl- β -D-glucopyranoside ³
Gentiobiose	O - β -D-glucopyranosyl-(1 \rightarrow 6)-D-glucopyranose ³
Melezitose	O - α -D-glucopyranosyl-(1 \rightarrow 3)- O - β -D-fructofuranosyl-(2 \rightarrow 1)- α -D-glucopyranoside ⁴
3- α -Isomaltosylglucose	O - α -D-glucopyranosyl-(1 \rightarrow 6)- O - α -D-glucopyranosyl-(1 \rightarrow 3)-D-glucopyranose ⁴
Maltotriose	O - α -D-glucopyranosyl-(1 \rightarrow 4)- O - α -D-glucopyranosyl-(1 \rightarrow 4)-D-glucopyranose ⁴
1-Kestose	O - α -D-glucopyranosyl-(1 \rightarrow 2)- β -D-fructofuranosyl-(1 \rightarrow 2)- β -D-fructofuranoside ⁴
Panose	O - α -D-glucopyranosyl-(1 \rightarrow 6)- O - α -D-glucopyranosyl-(1 \rightarrow 4)-D-glucopyranose ⁴
Isomaltotriose	O - α -D-glucopyranosyl-(1 \rightarrow 6)- O - α -D-glucopyranosyl-(1 \rightarrow 6)-D-glucopyranose ⁴
Erlose	O - α -D-glucopyranosyl-(1 \rightarrow 4)- α -D-glucopyranosyl- β -D-fructofuranoside ⁴
Theanderose	O - α -D-glucopyranosyl-(1 \rightarrow 6)- α -D-glucopyranosyl- β -D-fructofuranoside ⁴
Centose	O - α -D-glucopyranosyl-(1 \rightarrow 4)- O - α -D-glucopyranosyl-(1 \rightarrow 2)-D-glucopyranose ⁴
Isopanose	O - α -D-glucopyranosyl-(1 \rightarrow 4)- O - α -D-glucopyranosyl-(1 \rightarrow 6)-D-glucopyranose ⁴
Isomaltotetraose	O - α -D-glucopyranosyl-(1 \rightarrow 6)-[O - α -D-glucopyranosyl-(1 \rightarrow 6)] ₂ -D-glucopyranose ⁴
Isomaltopentaose	O - α -D-glucopyranosyl-(1 \rightarrow 6)-[O - α -D-glucopyranosyl-(1 \rightarrow 6)] ₃ -D-glucopyranose ⁴

¹ Source: White and Hoban (1959).² Source: Watanabe and Aso (1959).³ Source: Siddiqui and Furgala (1967).⁴ Source: Siddiqui and Furgala (1968).

This material was further separated into 15 portions from a charcoal-celite column, using aqueous ethanol (0–30%) in a stepwise elution. These 15 fractions were subjected to paper chromatography and paper electrophoresis, allowing each sugar to be obtained pure. A variety of physical and chemical methods were used to characterize these sugars: partial acid and enzyme hydrolysis, comparison of specific rotation of the sugar and its acetate with standards, and comparison of the chromatographic mobilities of the sugars and their acetates with standards. The total yield of these sugars from honey amounted to just 3.65%, and the yield of each is given in Table 7.11. Recent advances in separating sugar mixtures, such as HPLC and GLC, are not adequate for resolving the complex mixture found in honey.

TABLE 7.11. YIELDS OF THE PRINCIPAL SUGARS IN THE OLIGOSACCHARIDE FRACTION (3.65%) OF HONEY

Disaccharides	%	Trisaccharides	%	Higher saccharides	%
Maltose	29.4	Erlose	4.5	Isomaltotetraose	0.1
Kojibiose	8.2	Theanderose	2.7	Isomaltopentaose	0.1
Turanose	4.7	Panose	2.5		
Isomaltose	4.4	Maltotriose	1.9		
Sucrose	3.9	1-Kestose	0.9		
Maltulose (and two unidentified ketoses)	3.1	Isomaltotriose	0.6		
Nigerose	1.7	Melezitose	0.6		
α , β -Trehalose	1.1	Isopanose	0.24		
Gentiobiose	0.4	Centose	0.05		
Laminaribiose	0.09	3- α -Isomaltosyl-Glucose	trace		
Total	56.99		13.69		0.1

Source: Siddiqui (1970).

Crystallization of Honey

Nearly all honeys are supersaturated with respect to glucose; the exceptions are noncrystallizing honey types which are low in glucose, such as tupelo. Glucose crystallizes from honey as its monohydrate, and while this happens only rarely while the honey is in the comb, commonly occurs after extraction. This is due to a considerable extent to the lower storage temperature to which honey is exposed after extraction. By heating and filtering honey to remove seed crystals of glucose monohydrate, crystallization can be delayed many months.

The major problem which results from crystallization is that the resulting increased moisture content of the liquid phase above the crystals allows osmophilic yeasts to ferment the honey. Such yeasts are present in bees and nectar, and therefore, are nearly ubiquitous in honeys. Considerable efforts have been directed toward preventing crystallization of honey, and indices have been evaluated so that one may predict whether a given honey will tend to crystallize. To this end, the glucose: water ratio in a particular honey is useful, since it gives a highly significant relationship to crystallizing tendency. Glucose: water ratios of 1.70 and lower are generally associated with noncrystallizing honey and values of 2.10 and higher predict rapid crystallization. Problems associated with the crystallization of honey have been detailed (White 1978).

Nutritive Value of Honey

Folklore regarding honey dates back 5000 years. There may be no area in which scientific opinion clashes with folklore more than in the nutritive and medical aspects of honey. Over 2000 articles have been published on the subject since the Second World War. A chapter (Anon

1975) on the biological properties of honey appeared in a book on honey, which provided a consensus of six authorities on the subject.

Honey is a nutritive sweetener, because of its high levels of glucose and fructose and its variable content of trace minerals. The vitamin content has no nutritional significance, since in most honeys their level is orders of magnitude less than the recommended daily intake. Because of its high levels of glucose and fructose, honey is an excellent source of energy, as these sugars are completely absorbed in the small intestine. Honey therefore is first and foremost to be valued as a natural sweetener with an infinite variety of flavors and aromas, which cannot be found elsewhere. It is primarily a food for bees, not man, and even the bees must supplement it with quantities of proteinaceous pollen.

Quality Assurance of Honey

The qualities of honey which are normally monitored by food manufacturers include moisture, flavor, color, cleanliness, and authenticity. Moisture is determined by refractometry, and color is evaluated using a Pfund Honey Color Grader.

The relatively high prices, limited supply, and complexity of honey combine to offer incentives for adulteration with inexpensive, highly refined sweeteners. Early adulterants included corn syrup and inverted sucrose syrups derived from sugar cane and sugar beets. Suitable methods are available for the detection of these syrups in honey (Horwitz 1980). Corn syrup is mainly glucose, so when it is mixed with honey, the level of this sugar will exceed that normally found in honey. Acid-inverted sucrose syrups contain higher levels of hydroxymethylfurfural (HMF) than are normally found to occur naturally in honey. Samples with HMF values over 20 mg/100 g can be suspected of being adulterated.

Since the advent of high fructose corn syrup (HFCS), there has been concern that it is being used to adulterate honey. HFCS is produced by isomerizing a portion of the glucose in conventional corn syrup with the enzyme glucose isomerase. These syrups are relatively inexpensive and contain fructose and glucose in proportions within the range found in honeys. HFCS is highly refined and contains only small quantities of higher saccharides. A thin-layer chromatographic method has been developed (Kushnir 1979) in which adulteration can be indicated by the measure of low R_f spots corresponding to the trace HFCS higher saccharides, probably glucans.

A definitive test for HFCS adulteration of honey has been developed (White and Doner 1978) based on mass spectrometric determination of $^{13}\text{C}/^{12}\text{C}$ ratios. This method is noncircumventible and will detect the presence of any corn or cane derived syrup. The method takes advantage

of the fact that the isotopes of carbon in carbon dioxide are fractionated to different degrees during photosynthesis via the two main pathways, the Calvin cycle and the Hatch-Slack cycle. Hatch-Slack plants, such as sugar cane and corn, discriminate to a lesser degree against ^{13}C atmospheric carbon dioxide than do Calvin plants, and as a result, organic compounds in sugar cane and corn are "heavier" in ^{13}C than the carbon compounds from Calvin plants.

United States honey samples (84), representing all commercial significant floral sources, were analyzed for $^{13}\text{C}/^{12}\text{C}$ ratios. Also, ratios for 35 imported samples from 15 countries and 4 HFCS samples were determined. It was found (Doner and White 1977) that values for all honey samples were in the range associated with Calvin plants. A statistical analysis of the $\delta^{13}\text{C}$ data appears in Table 7.12 and distribution of values in Fig. 7.11. The $^{13}\text{C}/^{12}\text{C}$ ratios of a sample reported as per mil (‰) deviations from a limestone standard and defined as

$$\delta^{13}\text{C} (\text{‰}) = \left[\frac{^{13}\text{C}/^{12}\text{C} \text{ sample}}{^{13}\text{C}/^{12}\text{C} \text{ standard}} - 1 \right] \times 10^3$$

There appears to be no correlation between $\delta^{13}\text{C}$ value and source of honey; results for samples from 17 plant families are in Table 7.11.

The coefficient of variation for all honey samples was 3.86%, the smallest yet encountered for any constituent or physical property of honey. Mixtures of HFCS and honey have $\delta^{13}\text{C}$ values equal to the sum of the fractional contribution of each. The $\delta^{13}\text{C}$ values of a sample provide the basis for a method to detect mixtures. This method was sanctioned Official Final Action by the Association of Official Analytical Chemists and is widely used by regulatory agencies and by the honey industry for self-policing.

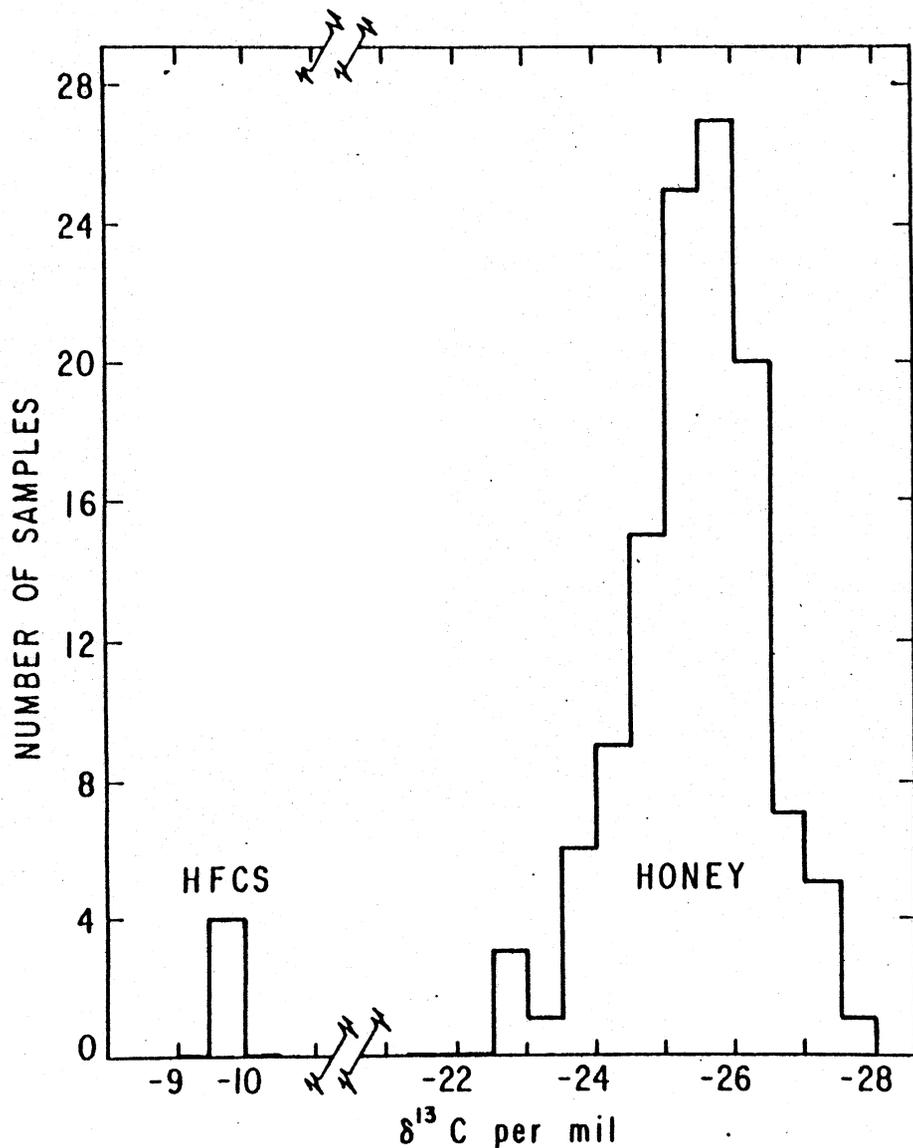
TABLE 7.12. $\delta^{13}\text{C}$ VALUES OF HONEYS AND HIGH FRUCTOSE CORN SYRUP (HFCS)

Source	No. of samples	Mean (‰)	Range		S.D. (‰)	Coef. of variation (%)
			High	Low		
United States honey	84	-25.2	-22.6	-27.4	0.94	3.86
Imported honey	35	-25.8	-23.9	-27.4	0.97	3.86
All honey	119	-25.4	-22.5	-27.4	0.98	3.86
HFCS	4	-9.7	-9.5	-9.8	0.14	1.43

Source: White and Doner (1978).

Future Research Needs

A fundamental question which remains to be answered concerns the mechanism of formation of many of the di- and trisaccharides in honey.



From White and Doner (1978)

FIG. 7.11. DISTRIBUTION OF $\delta^{13}\text{C}$ VALUES AMONG 119 SAMPLES OF HONEY AND 4 SAMPLES OF HIGH FRUCTOSE CORN SYRUP

TABLE 7.13. $\delta^{13}\text{C}$ VALUES FOR HONEY SAMPLES FROM ALL SIGNIFICANT FLORAL SOURCES

Family	Floral type	Number of samples	$\delta^{13}\text{C}$ (per mil)
Anacardiaceae	<i>Schinum molle</i> (pepper tree)	1	-21
Aquifoliaceae	<i>Ilex glabra</i> (gallberry)	1	-21
Compositae	<i>Centauria solstitialis</i> (star thistle)	1	-21
Cornaceae	<i>Nyssa ogeche</i> (tupelo)	1	-21
Cyrillaceae	<i>Cyrilla parvifolia</i> (titi)	1	-21
Euphorbiaceae	<i>Sapium sebiferum</i> (tallow tree)	1	-21
Labiatae	<i>Salvia</i> spp. (sage)	1	-21
Leguminosae	<i>Glycine soja</i> (soybean)	2	-21
Leguminosae	<i>Medicago sativa</i> (alfalfa)	10	-21
Leguminosae	<i>Melilotus</i> spp. (sweet clover)	2	-21
Leguminosae	<i>Trifolium</i> spp. (clover)	11	-21
Magnoliaceae	<i>Liriodendron tulipifera</i> (tulip tree)	1	-21
Malvaceae	<i>Gossypium hisutum</i> (cotton)	1	-21
Onagraceae	<i>Epilobium angustifolium</i> (fireweed)	1	-21
Palmae	<i>Sabal</i> spp. (palmetto)	1	-21
Polygonaceae	<i>Fagopyrum esculentum</i> (buckwheat)	1	-21
Rosaceae	<i>Rubus</i> spp. (blackberry)	1	-21
Rutaceae	<i>Citrus</i> spp. (orange, grapefruit)	3	-21
Tamaricaceae	<i>Tamarix gallica</i> (tamarisk)	1	-21
Tiliaceae	<i>Tilia americana</i> (basswood)	1	-21
	Unclassified natural season blends	15	-21
	Honeydew honey	4	-21

Source: Doner and White (1977).

Enzyme activities have not yet been identified to account for the maturation of most of these sugars, and it has not been demonstrated they can be produced by reversion from the simple sugars glucose and fructose.

It is essential for the integrity of honey markets that when commercial syrups become available, methods be developed to detect them in admixture with honey, so that falsification is discouraged.

Finally, to maintain and strengthen markets for honey, unique attributes of this natural sweetener which cannot be duplicated by expensive refined sweetening agents must be identified. A strong honey market is essential, since it not only provides a large portion of the beekeepers income, but billions of dollars worth of crops depend on the honeybee for pollination.

MAPLE SUGAR

Introduction

Of the 13 species of maple (*Acer*) native to North America, only the sugar maple (*Acer saccharum* Marsh.) and the black maple (*A. nigrum* Michx F.) are important in maple syrup production. These two species are favored primarily because their sap is sweeter than

from other species found in our hardwood forests, including the red maple (*Acer rubrum* L.) and the silver maple (*Acer saccharinum* L.) The region of maple syrup production extends from Maine west to Minnesota and from Quebec south to Indiana and West Virginia.

It is likely that the Indians living in the area of the Great Lakes and the St. Lawrence River were the first to produce maple syrup and sugar, as these products were found to be well established items of barter among the Indians by the first settlers. The settlers quickly recognized the value of maple sugar and, with cane sugar being expensive and difficult to obtain, learned how to make it themselves.

In early 18th Century colonial America, with tea and coffee being consumed in greater amounts, maple sugar was increasingly relied upon as a sweetener. The total production of maple products (listed as syrup equivalent) in the United States and Canada from 1850 until recently is given in Table 7.14. Because of increased supplies of cane and beet sugars, the United States production has steadily declined. Production in Quebec alone now more than doubles United States production, and sizable quantities are imported from Canada.

TABLE 7.14. PRODUCTION OF MAPLE SYRUP IN THE UNITED STATES AND CANADA (IN THOUSAND GALLONS)

Year	United States	Canada
1870	4477	2160
1890	6377	3136
1909	5859	3476
1929	2509	2385
1949	1480	2608
1959	1049	3092
1971	962	1676
1972	1099	2470
1973	857	2988
1974	1087	2182

Source: Raymond and Winch (1969); Agricultural Statistics (1978).

Composition of Maple Sap

Maple sap as it comes from the tree, contains about 2% solids, of which sucrose constitutes about 97% (Willits and Hills 1976). The remainder of the solids includes organic acids (1.5%), ash (0.7%), nitrogenous material (0.4%), quebrachitol (Stinson *et al.* 1967), polysaccharide, and trace amounts of lignin and related compounds (Filipic and Underwood 1964). Malic acid accounts for about 90% of the organic acid fraction, with citric, succinic, fumaric, and several unidentified acids also present (Porter *et al.* 1951). The nitrogenous fraction has been shown (Pol-

lard and Sproston 1954) to contain ammonia and protein, but no free amino acids are present until the tree breaks dormancy. The ligneous fraction contains coumarin, vanillin, syringaldehyde, coniferylaldehyde, 2,6-dimethoxybenzoquinone, and a higher molecular weight lignin. Even though these latter compounds are present in sap in just trace amounts, they contribute to the flavor of maple syrup.

Early reports suggested that small quantities of invert sugar are present in fresh maple sap, but when precautions are taken to prevent bacterial contamination, no glucose or fructose are found (Willits and Hills 1976; Haq and Adams 1961).

Two groups have examined maple sap for the presence of oligosaccharides, using a preliminary fractionation by charcoal-celite chromatography. In 1954 (Porter *et al.*), a deionized sap concentrate was fractionated by this method, to yield an oligosaccharide fraction essentially free from sucrose. Preparative paper chromatography of this fraction revealed the presence of five bands, one of which was eluted from the paper and shown by paper electrophoresis to consist of two components. Characterization of the fragments produced by acid hydrolysis and by incubation with invertase and melibiase suggested that one of the sugars was raffinose and the other a "glucosylsucrose."

In a later study (Haq and Adams 1961), raffinose was not found but neokestose [O - β -D-fructofuranosyl-(2 \rightarrow 6)- α -D-glucopyranosyl-(1 \rightarrow 2)- β -D-fructofuranoside] was confirmed to be present. Charcoal-celite chromatography followed by preparative paper chromatography accomplished the isolation of this sugar in a pure state, and it was characterized by enzymatic hydrolysis, partial and total acid hydrolysis, and methylation analysis. A small quantity of another sugar was isolated and similar methods of analysis suggested it was also a fructosylsucrose, either 1-kestose [O - α -D-glucopyranosyl-(1 \rightarrow 2)- β -D-fructofuranosyl-(1 \rightarrow 2)- β -D-fructofuranoside] or [O - α -D-glucopyranosyl-(1 \rightarrow 2)- β -D-fructofuranosyl-(1 \rightarrow 6)- β -D-fructofuranoside]. Earlier, cellobiose had been reported to be present in maple sap, but it was found in neither of these detailed analyses.

Composition of Maple Syrup

An analysis of two samples of Japanese maple syrup by paper chromatography after a preliminary charcoal-celite column fractionation was conducted (Watanabe and Aso 1962). On the basis of R_f values and reaction to spray reagents, the following sugars were reported to be present: sucrose (80–83% of total sugars), glucose (4%), fructose (3%), xylose, arabinose, galactose, melezitose, and eight or nine oligosaccharides containing a ketose, none of which was isolated and characterized.

It is possible that arabinose and galactose had their origin in the arabinogalactan that had been isolated earlier from maple sap (Adams and Bishop 1960).

There exists a need for a survey of additional samples of maple sap and syrup to determine whether there is a variability in the oligosaccharide content and to establish if the trisaccharides raffinose and neokestose are always present in sterile sap. There are obviously many chemical changes that occur during the transformation from sap into syrup, notably the formation of glucose and fructose from sap in which these sugars were absent. The acidic pH early in the evaporation process will cause some sucrose inversion, and the alkaline pH (eight to nine) later could bring about further changes in the sugar content. If sucrose hydrolysis occurs, it is likely that sap trisaccharides would also be effected. Alkaline degradation reactions of sugars occur during the syrup-making process, since glyceraldehyde, methyl glyoxal, a reductone, and acetol have been found in the steam distillate of maple syrup (Underwood *et al.* 1956).

Quality Assurance of Maple Syrup

Most consumers of maple products purchase syrup that has low levels of maple syrup and greater levels of cane or beet syrup. Such products must be appropriately labeled as to their composition. As with honey, maple syrup has traditionally been a target for adulteration. Since pure maple syrup is largely sucrose, inexpensive syrups of this sugar are sometimes mixed with maple and labeled as pure.

The stable carbon isotope ratio analysis (SCIRA) method, described earlier for honey adulteration, has been demonstrated (Hillaire-Marcel *et al.* 1977; Carro *et al.* 1980) to be effective in detecting adulteration of maple products with cane sucrose. Whereas sucrose averages about -11.3% in $\delta^{13}\text{C}$, pure maple syrups average about -23.8% , allowing the distinction between pure and adulterated syrups.

BIBLIOGRAPHY

- ADAMS, G.A. and BISHOP, C.T. 1960. Constitution of an arabinogalactan from maple sap. *Can. J. Chem.* 38, 2380-2386.
- AGRICULTURAL STATISTICS. 1978. U.S. Department of Agriculture, Washington, DC.
- ANON. 1975. Biological properties of honey. In *Honey: A Comprehensive Survey*. E. Crane (Editor). Heinemann Publishing Co., London.
- ANON. 1979A. Workshop discussion on lactose crystallization and lactose hydrolysis. *N. Z. J. Dairy Sci. Technol.* 14, 128-130.

- ANON. 1979B. Hydrolyzed lactose: New source of sweeteners. *Food Eng.* 51 (11), 30-31.
- ANON. 1979C. Can a new laxative beat the habit? *Chem. Week* 125 (17) 50-51.
- BAER, R.J., FRANK, J.F., and LOEWENSTEIN, N. 1980. Freezing point measurements of lactose hydrolysis in acid whey and lactose solutions. *J. Assoc. Off. Anal. Chem.* 63, 587-590.
- BUMA, T.J. and WIEGERS, G.A. X Ray powder patterns of lactose and unit cell dimensions of β -lactose. 1967. *Neth. Milk Dairy J.* 21, 208-213.
- CARRO, O., HILLAIRE-MARCEL, C., and GAGNON, M. 1980. Detection of adulterated maple products by stable carbon isotope ratio. *J. Assoc. Off. Anal. Chem.* 63, 840-844.
- CARUBELLI, R. 1966. Transformation of disaccharides during borate ion exchange chromatography. Isomerization of lactose into lactulose. *Carbohydr. Res.* 2, 480-485.
- CHIRIFE, J. and FERRO-FONTAN, C.F. 1980. Water activity of aqueous lactulose solutions. *J. Food Sci.* 45, 1706-1707.
- CLARK, W.S. JR. 1979. Symposium on the chemical and nutritional aspect of dairy wastes. Introduction. *J. Agric. Food Chem.* 27, 653-654.
- CONN, H.O. 1978. Lactulose: A drug in search of a modus operandi. *Gastroenterology* 74, 624-626.
- CORBETT, W.M. and KENNER, J. 1954. The degradation of carbohydrate by alkali. Part V. Lactulose, maltose, and maltulose. *J. Chem. Soc.* 1789-1791.
- DAHLQVIST, A. and GRYBOSKI, J.D. 1965. Inability of the human small intestinal lactase to hydrolyze lactulose. *Biochim. Biophys. Acta* 110 635-636.
- DELBEKE, R. 1979. Purification of an ultrafiltration permeate with adsorbant and ion-exchange resins. *Neth. Milk Dairy J.* 33, 181-192.
- DEVRIES J.W., HEROFF, J.C., and EGBERG, D.C. 1979. High pressure liquid chromatographic determination of carbohydrates in food products. Evaluation of method. *J. Assoc. Off. Anal. Chem.* 62, 1292-1296.
- DONER, L.W. 1977. The sugars of honey—A review. *J. Sci. Food Agric.* 28 443-456.
- DONER, L.W. and WHITE, J.W. JR. 1977. Carbon-13/carbon-12 ratio is relatively uniform among honeys. *Science* 197, 891-892.
- DONER, L.W., WHITE, J.W. JR., and PHILLIPS, J.G. 1979. Gas-liquid chromatographic test for honey adulteration by high fructose corn syrup. *J. Assoc. Off. Anal. Chem.* 62, 186-189.
- EUBER, J.R. and BRUNNER, J.R. 1979. Determination of lactose in milk products by high performance liquid chromatography. *J. Dairy Sci.* 62 685-690.
- FILIPIC, V.J. and UNDERWOOD, J.C. 1964. Compositions of maple sap and sirup - some aromatic compounds in sap. *J. Food Sci.* 29, 464-468.
- GUTH, J.H. and TUMMERMAN, L. 1970. Method of making lactulose U.S. Pat. 3,546,206. Dec. 8.

- GUY, E.J. 1978. Evaluation of milk caramels containing hydrolyzed lactose. *J. Food Sci.* **43**, 980-984.
- GUY, E.J. 1980. Partial replacement of nonfat milk solids and cane sugar in ice cream with lactose hydrolyzed sweet whey solids. *J. Food Sci.* **45**, 129-133.
- GUY, E.J. and EDMONDSON, L.F. 1978. Preparation and properties of sirups made by hydrolysis of lactose. *J. Dairy Sci.* **61**, 542-549.
- HANSEN, R.G. and GITZELMANN, R. 1975. The metabolism of lactose and galactose. *In Physiological Effects of Food Carbohydrates*. A. Jeanes and J. Hodge (Editors). ACS Symp. Ser. 15, American Chemical Society, Washington, DC.
- HAQ, S. and ADAMS, G.A. 1961. Oligosaccharides from the sap of sugar maple (*Acer saccharum* Marsh.). *Can. J. Chem.* **39**, 1165-1170.
- HARJU, M. and KREULA, M. 1980. Lactose hydrolysates. *In Carbohydrate Sweeteners in Foods and Nutrition*. P. Koivistoinen and L. Hyvönen (Editors). Academic Press, New York.
- HARPER, W.J. 1979. Review — Analytical procedures for whey and whey products. *N. Z. J. Dairy Sci. Technol.* **14**, 156-171.
- HAYASHIBARA, K. and SUGIMOTO, K. 1976. Containing lactitol as a sweetener. U.S. Pat. 3,973,050. Aug. 3.
- HICKS, K.B. and PARRISH, F.W. 1980. A new method for the preparation of lactulose from lactose. *Carbohydr. Res.* **82**, 393-397.
- HILLAIRE-MARCEL, C., CARRO-JOST, O., and JACOB, C. 1977. Composition of the isotopes $^{13}\text{C}/^{12}\text{C}$ in sucrose and glucose of various sources for control of maple sugar and syrups. *J. Inst. Can. Sci. Technol. Aliment.* **10**, 333-335.
- HORWITZ, W. (Editor). 1980. Official Methods of Analysis of the Association of Official Analytical Chemists, 13th edition. Washington, DC.
- HOUGH, L., JONES, J.K.N., and RICHARDS, E.L. 1953. The reaction of amino-compounds with sugars. Part II. The action of ammonia on glucose, maltose, and lactose. *J. Chem. Soc.* 2005-2009.
- HUHTANEN, C.N., PARRISH, F.W., and HICKS, K.B. 1980. Inhibition of bacteria by lactulose preparations. *Appl. Environ. Microbiol.* **40**, 171-173.
- HURST, W.J. and MARTIN, R.A. JR. 1980. High performance liquid chromatographic determination of carbohydrates in chocolate: Collaborative study. *J. Assoc. Off. Anal. Chem.* **63**, 595-599.
- HURST, W.J., MARTIN, R.A. JR., and ZOUMAS, B.L. 1979. Application of hplc to characterization of individual carbohydrates in foods. *J. Food Sci.* **44**, 892-895.
- ISBELL, H.S. and PIGMAN, W.W. 1938. Pyranose furanose interconversions with reference to the mutarotations of galactose, levulose, lactulose, and turanose. *J. Res. Natl. Bur. Stand.* **20**, 773-798.
- KINOSHITA, J.H. 1974. Cataractogenic effects of lactose and galactose. *In Sugars in Nutrition*. H.L. Sipple and K.W. McNutt (Editors). Academic Press, Inc., New York.
- KUSHNIR, I. 1979. Sensitive thin layer chromatographic detection of high fructose corn sirup in honey. *J. Assoc. Off. Anal. Chem.* **62**, 917-920.
- LIM, S.G. and NICKERSON, T.A. 1973. Effect of methanol on the various forms of lactose. *J. Dairy Sci.* **56**, 843-848.
- LIN, A.Y. and NICKERSON, T.A. 1977. Acid hydrolysis of lactose in whey versus aqueous solutions. *J. Dairy Sci.* **60**, 34-39.
- LINKO, P., SAIJONMAA, T., HEIKONEN, M., and KREULA, M. 1980. Lactitol. *In Carbohydrate Sweeteners in Food and Nutrition*. P. Koivistoinen and L. Hyvönen (Editors). Academic Press, New York.
- MACBEAN, R.D., HALL, R.J., and WILLMAN, N.J. 1979. Heterogeneous acid-catalyzed hydrolysis of lactose with cation exchange resins. *Aust. J. Dairy Technol.* **34**, 53-59.
- MADJ, F. and NICKERSON, T.A. 1976. Effect of alcohols on lactose solubility. *J. Dairy Sci.* **59**, 1025-1032.
- MAKINEN, K.K. and REKOLA, M. 1975. Comparison between sucrose and lactulose in a suspended salivary system. *J. Dent. Res.* **54**, 1244.
- MARVIN, J.W., BERNHARD, R.A., and NICKERSON, T.A. 1979. Interactions of low molecular weight adsorbates on lactose. *J. Dairy Sci.* **62**, 1546-1557.
- MATTHEWS, M.E. 1979. Advances in whey processing-ultrafiltration and reverse osmosis. *N. Z. J. Dairy Sci. Technol.* **14**, 86-92.
- MAURIZIO, A. 1975. How bees make honey. *In Honey: A Comprehensive Survey*. E. Crane (Editor). Heinemann Publishing Co., London.
- MAYO, J.A. 1981. Personal Communication. New Orleans, LA.
- MC COMMINS, D.B., BERNHARD, R.A., and NICKERSON, T.A. 1980. Recovery of lactose from aqueous solutions: Precipitation with calcium hydroxide and sodium hydroxide. *J. Food Sci.* **45**, 362-366.
- MENDEZ, A. and OLANO, A. 1979. Lactulose. A review of some chemical properties and application in infant nutrition and medicine. *Dairy Sci. Abst.* **41**, 531-535.
- MENDICINO, J.F. 1960. Effect of borate on the alkali-catalyzed isomerization of sugars. *J. Am. Chem. Soc.* **82**, 4975-4979.
- MONTGOMERY, E.M. and HUDSON, C.S. 1930. Relations between rotary power and structure in the sugar group. XXVII. Synthesis of a new disaccharide ketose (lactulose) from lactose. *J. Am. Chem. Soc.* **52**, 2101-2106.
- MOORE, K. 1980. Immobilized enzyme technology commercially hydrolyzes lactose. *Food Prod. Dev.* **14** (1), 50-51.
- NEZBED, R.L. 1974. Amorphous beta lactose for tableting. U.S. Pat. 3,802,914. April 9.
- NICKERSON, T.A. 1974. Lactose. *In Fundamentals of Dairy Chemistry*. B.H. Webb, A.H. Johnson, and J.A. Alford (Editors). AVI Publishing Co., Westport, CT.
- NICKERSON, T.A. 1979. Lactose chemistry. *J. Agric. Food. Chem.* **27**, 672-677.
- NICKERSON, T.A. and LIM S.G. 1974. Effect of various alcohols on lactose. *J. Dairy Sci.* **57**, 1320-1324.

- OLANO, A. 1978. Treatment of forms of lactose with dilute alcoholic solutions of sodium hydroxide. *J. Dairy Sci.* 61, 1622-1623.
- OLANO, A., NICKERSON, T.A., and BERNHARD, R.A. 1977A. Recovery of lactose from aqueous solutions: Precipitation in the presence of calcium hydroxide and ammonium chloride additions. *J. Food Sci.* 42, 1481-1483.
- OLANO, A., NICKERSON, T.A. and BERNHARD, R.A. 1977B. Recovery of lactose from aqueous solutions: Precipitation with calcium chloride and sodium hydroxide. *J. Food Sci.* 42, 1484-1486.
- OLANO, O. and RIOS, J.J. 1978. Treatment of lactose with alkaline methanolic solutions: Productions of beta-lactose from alpha-lactose hydrate. *J. Dairy Sci.* 61, 300-302.
- OOSTEN, B.J. 1967A. Solubility diagram of lactose and lactulose in water. *Rec. Trav. Chim.* 86, 675-676.
- OOSTEN, B.J. 1967B. Crystallization of lactulose. *Rec. Trav. Chim. Pays-Bas* 86, 673-674.
- PAIGE, D.M., BAYLESS, T.M., HUANG, S., and WEXLER, R. 1975. Lactose intolerance and lactose hydrolyzed milk. In *Physiological Effects of Food Carbohydrates*. A. Jeahes and J. Hodge (Editors). ACS Symp. Ser. 15, American Chemical Society, Washington, DC.
- PARRISH, F.W. 1981. Personal communication. New Orleans, LA.
- PARRISH, F.W., SHARPLES, P.M., HOAGLAND, P.D., and WOYCHIK, J.H. 1979A. Demineralization of cheddar whey ultrafiltrate with thermally regenerable ion-exchange resins: Improved yield of α -lactose monohydrate. *J. Food Sci.* 44, 555-557.
- PARRISH, F.W., PFEFFER, P.E., ROSS, K.D., SCHWARTZ, D.P., VALENTINE, K.M. 1979B. Retention of Aliphatic Alcohols by Anhydrous Lactose. *J. Agric. Food Chem.* 27, 56-59.
- PARRISH, F.W., TALLEY, F.B., ROSS, K.D., CLARK, J., and PHILLIPS, J.G. 1979C. Sweetness of lactulose relative to sucrose. *J. Food Sci.* 44, 813-815.
- PARRISH, F.W., ROSS, K.D., and SIMPSON, T.D. 1979D. Formation of β -lactose from α - and β -lactose octaacetates, and from α -lactose monohydrate. *Carbohydr. Res.* 71, 322-326.
- PARRISH, F.W., ROSS, K.D., and VALENTINE, K.M. 1980A. Formation of β -lactose from the stable forms of anhydrous α -lactose. *J. Food Sci.* 45, 68-70.
- PARRISH, F.W., HICKS, K.B., and DONER, L.W. 1980B. Analysis of lactulose preparations by spectrophotometric and high performance liquid chromatographic methods. *J. Dairy Sci.* 63, 1809-1814.
- PERLIN, A.S., HERVE DU PENHOAT, P., and ISBELL, H.S. 1973. Carbon-13 and hydroxyl proton nmr spectra of ketoses. A conformational and compositional description of ketohexoses in solution. In *Carbohydrates in Solution*. Advances in Chemistry Ser. 117. R.F. Gould (Editor). American Chemical Society, Washington, DC.
- PFEFFER, P.E. and HICKS, K.B. 1980. Solution differential isotope shift and solid state cross polarization ^{13}C NMR spectroscopy of lactulose. Paper 26, Carbohydr. Div. Abstracts of Papers, 179th ACS National Meeting.
- POLLARD, J.K. and SPROSTON, T. 1954. Nitrogenous constituents from the sapwood of *Acer saccharum*. *Plant Physiol.* 29, 360-364.
- PORTER, W.L., BUCH, M.L., and WILLITS, C.O. 1951. Maple sirup. III. Preliminary study of the nonvolatile acid fraction. *Food Res.* 16, 338-341.
- PORTER, W.L., HOBAN, N., and WILLITS, C.O. 1954. Contribution to the chemistry of maple sap and syrup. *Food Res.* 19, 597-602.
- RAYMOND, L.S. and WINCH, F.E. 1969. New York State College of Agriculture Bulletin. Cornell University, Ithaca, NY.
- RENDELMAN, J.A. JR. and HODGE, J.E. 1979. Complexes of carbohydrates with aluminate ion. Aldose-ketose interconversion on anion-exchange resin (aluminat and hydroxide forms). *Carbohydr. Res.* 75, 83-99.
- ROSS, K.D. 1978. Effects of methanol on physical properties of α - and β -lactose. *J. Dairy Sci.* 61, 152-158.
- ROSS, K.D., PARRISH, F.W., and HUHTANEN, C.N. 1979. Derivatives of lactose for controlling water activity. Paper No. 280, 39th Annual IFT Meeting.
- SAIJONMAA, T., HEIKONEN, M., KREULA, M., and LINKO, P. 1978. Preparation and characterization of milk sugar alcohol, lactitol. *Milchwissenschaft* 33, 733-736.
- SHUKLA, T.P. 1975. Beta-galactosidase technology: A solution to the lactose problem. *CRC Crit. Rev. Food Technol.* 5, 325-356.
- SIDDIQUI, I.R. 1970. The sugars of honey. *Adv. Carbohydr. Chem. Biochem.* 25.
- SIDDIQUI, I.R. and FURGALA, B. 1967. Isolation and characterization of oligosaccharides from honey. Part I. Disaccharides. *J. Apic. Res.* 6, 139-145.
- SIDDIQUI, I.R. and FURGALA, B. 1968. Isolation and characterization of oligosaccharides from honey. Part II. Trisaccharides. *J. Apic. Res.* 7, 51-59.
- SPECK, J.C. JR. 1958. The Lobry de Bruyn-Alberda van Ekenstein transformation. *Adv. Carbohydr. Chem.* 13, 63-103.
- STINSON, E.E., DOOLEY, C.J., PURCELL, J.M., and ARD, J.S. 1967. Quebrachitol—A new component of maple sap and syrup. *J. Agric. Food Chem.* 15, 394-397.
- THEAN, J.E. and FUNDERBURK, W.C. JR. 1977. High pressure liquid chromatographic determination of sucrose in honey. *J. Assoc. Off. Anal. Chem.* 60, 838-841.
- THELWALL, L.A.W. 1980. Lactose. In *Developments in Food Carbohydrates - 2*. Developments Series. Applied Science Publishers, Ltd., London.
- UNDERWOOD, J.C., LENTO, H.G. JR., and WILLITS, C.O. 1956. Triosaccharides in maple sirup. *Food Res.* 21, 589-597.
- VAN VELTHUIJSEN, J.A. 1979. Food additives derived from lactose: Lactitol and lactitol palmitate. *J. Agric. Food Chem.* 27, 680-686.
- VUJICIC, I.F., LIN, A.Y., and NICKERSON, T.A. 1977. Changes during acid hydrolysis of lactose. *J. Dairy Sci.* 60, 29-33.

- WARTHESEN, J.J. and KRAMER, P.L. 1979. Analysis of sugars in milk and ice cream by high pressure liquid chromatography. *J. Food Sci.* **44**, 626-627.
- WATANABE, T. and ASO, K. 1959. Isolation of kojibiose from honey. *Nature* **183**, 1740.
- WATANABE, T. and ASO, K. 1962. On the sugar composition of maple syrup. *Tohoku J. Agric. Res.* **13**, 175-181.
- WHISTLER, R.L. and DURSO, D.F. 1950. Chromatographic separation of sugars on charcoal. *J. Am. Chem. Soc.* **72**, 677-679.
- WHITE, J.W. JR. 1978. Honey. *Adv. Food Res.* **24**.
- WHITE, J.W. JR. and DONER, L.W. 1978. Mass spectrometric detection of high-fructose corn syrup in honey by use of $^{13}\text{C}/^{12}\text{C}$ ratio: Collaborative study. *J. Assoc. Off. Anal. Chem.* **61**, 746-750.
- WHITE, J.W. JR. and HOBAN, N. 1959. Composition of honey. IV. Identification of the disaccharides. *Arch. Biochem. Biophys.* **80**, 386-392.
- WHITE, J.W. JR. and MAHER, J. 1954. Selective adsorption method for determination of the sugars of honey. *J. Assoc. Off. Anal. Chem.* **37**, 466-478.
- WHITE, J.W. JR., RIETHOF, M.L., SUBERS, M.H., and KUSHNIR, I. 1962. Composition of American Honeys. U.S. Dept. Agric., Tech. Bull. 1261, Washington, DC.
- WILLITS, C.O. and HILLS, C.H. 1976. Maple syrup producers manual. Agriculture Handbook No. 134. U.S. Dept. of Agriculture, Washington, DC.
- WOOD, P.J., SIDDIQUI, I.R., and WEISZ, J. 1975. Determination of glucose and fructose in honey. *J. Apic. Res.* **14**, 41-45.
- ZARB, J.M. and HOURIGAN, J.A. 1979. An enzymatic, cryoscopic method for the estimation of lactose in milk products. *Aust. J. Dairy Tech.* **34**, 184-186.

Sugar Dehydration Reactions

*Milton S. Feather*¹

INTRODUCTION

The purpose of this chapter is to present some information on carbohydrate dehydration reactions as they relate to, and overlap with, the issue of food quality. Almost all foods contain measurable quantities of carbohydrates, and, during processing, undergo at least limited heating. As a result, the carbohydrates undergo a certain amount of degradation. Probably the best known reaction in this area is the Maillard reaction, or "nonenzymatic browning." This reaction, although known for more than 100 years, is a complex of many reactions, most of which are poorly understood.

The most significant reactions involve amines (protein and amino acids) interacting with sugars to produce brown, polymeric pigments, low-molecular-weight ultraviolet absorbing compounds including various food flavor and aroma constituents. It is clear that sugars play an important role in the reaction, undergoing dehydration, fragmentation, and, perhaps many other as yet unknown, reactions. Again, the details of these reactions are poorly understood and we have little information concerning the role of sugar type, pH, water concentration, and amine