Table III. Comparison of Acetaldehyde Data Obtained by by High-Performance Liquid Chromatography (HPLC) for Two *S. lactis* Strains

organism	incubation time[a]	acetaldehyde[b] mean	RSD
S. lactis 60	3	1.29	10.4
	6	3.99	8.1
	9	4.53	7.9
S. lactis C2	3	0.56	15.4
	6	2.97	5.7
	9	2.93	4.6

[a] Incubation in Elliker broth (h at 32 °C). [b] Corrected for initial (0 time) data; means from triplicate steam distillates from duplicate growth trials (micrograms of acetaldehyde per milliliter); relative standard deviation (RSD, percent).

obtained by DNPH-HPLC had slightly higher precision than did MBTH data. The mean RSD (across incubation times) for DNPH-HPLC data was 2.4% compared to 4.6% for MBTH data from steam distillates.

Comparison of acetaldehyde data for two strains of *S. lactis* are presented in Table III. Data presented are means from triplicate steam distillates for duplicate growth trials. Slightly higher acetaldehyde levels were detected in *S. lactis* 60 compared to *S. lactis* C2. The RSD for these data ranged from 4.6 to 15.4%, which included error associated with distillation and DNPH reaction as well as between trial error.

The steam distillation and DNPH trapping and reaction techniques appear to be acceptable for acetaldehyde analysis in *S. lactis* cultures. It has been suggested that steam distillation could result in artifacts from alehyde formations due to Strecker degradation of amino acids (Lees and Jago, 1969). However, low data variability between distillates for cultures or for Elliker broth blanks would suggest that heating effects are minimal. Any background produced by heating may also be minimized by correction for broth blank data and for zero time values. The advantages of high recovery and speed of analysis by steam distillation compared to low-temperature purging may overcome any potential data variability induced by artifacts resulting from steam distillation. When one distillation apparatus and manual HPLC injection were used, an approximate rate of six samples per h was attained. This could be greatly improved with more automated modifications.

Registry No. Acetaldehyde, 75-07-0; acetaldehyde 2,4-dinitrophenylhydrazone, 1019-57-4.

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Protection of Vitamin C by Sugars and Their Hydrogenated Derivatives

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The oxidation of vitamin C was studied in unaerated solutions at 100 °C and aerated solutions at 20 °C, with and without the presence of sugars or their hydrogenated derivatives. All sugars and derivatives protected the vitamin under certain conditions, but there was no obvious difference between hydrogenated and unhydrogenated carbohydrates. In copper-catalyzed, rapidly oxidizing systems, the protective effect of carbohydrate was marked. These results are interpretable on the basis of complexing of copper by carbohydrates or their hydrogenated counterparts by which means the catalytic destruction of the vitamin may be prevented.

The oxidation of vitamin C in food systems is well-known (Barron et al., 1936; Birch and Parker, 1974; Dekker and Dickinson, 1940; Selman, 1976) to be catalyzed by copper and other metals with loss of vitamin potency. The reactions involved are the sequential formation of dehydroascorbic acid (DHA), diketogulonic acid (DKA), and ultimately oxalic acid. The biological effectiveness of the vitamin is lost with the disappearance of the initial L-xyloascorbic acid and DHA. The oxidation is also evidently pH dependent because most studies report greatest stability of the vitamin at low pH.

Foods containing vitamin C are usually characterized by a high carbohydrate content. It is therefore important to study the role of sugars, as natural ingredients of foods, on vitamin C stability.

Carbohydrates, both mono- and polysaccharides, are well-known to form complexes with minerals and other food components (Angyal, 1973; Gallali et al., 1978; Ren-

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dleman, 1966a,b, 1978a,b). Such complexes might be extensive in foods containing both vitamin C and large quantities of carbohydrate and might confer a degree of protection on the vitamin. Polysaccharides would be anticipated to possess greater complexing power than monosaccharides but might not necessarily possess the correct balance of solution properties for inclusion in food formulations. The glucose syrups, however, being mixtures of oligosaccharides of a wide range of degree of polymerization, constitute an important water-soluble vehicle for chelation of minerals. Their chelating power for iron has already been reported (Kearsley et al., 1979). The nutritional and physiological advantages of using glucose syrups for human consumption are also well established (Kearsley et al., 1980).

Recently Briggs et al. (1981) have studied the complexing power of several carbohydrates and their derivatives by thin-layer ligand-exchange chromatography. Their studies have established the remarkable complexing power of the hydrogenated structures D-glucitol (sorbitol) and D-mannitol for copper. Sorbitol is already in use in foods as a diabetic sweetening agent. A recent U.K. Food Additives and Contaminants Committee report (1982), however, recommends the general use of sorbitol, mannitol, xylitol, isomalt, and hydrogenated glucose syrups in foods. The last mentioned substances are mixtures of sorbitol, maltitol, and higher reduced oligosaccharides. They are particularly stable in all food systems, do not participate in Maillard reactions, yet possess many of the structural features (polyhydroxy character) needed for mineral chelation.

This paper therefore describes experiments aimed at quantifying the protective effect of both hydrogenated and unhydrogenated sugars for vitamin C during copper-catalyzed oxidation.

EXPERIMENTAL SECTION

Materials. Commercial glucose syrups (18DE, 28DE, 55DE, 63DE, and hydrogenated 55DE glucose syrup) and maltitol were obtained from Roquette (U.K.), Ltd., Tunbridge Wells, Kent, England, as kind gifts. The 21DE glucose syrup was obtained from CPC, Manchester, U.K. Glucose, sorbitol, maltose, copper sulfate, L-ascorbic acid, and 2,6-dichlorophenolindophenol were purchased from Sigma (London) Chemical Co., Poole, Dorset, England. Nickel aluminum alloy, liquid paraffin, duolite, and metaphosphoric acid were obtained from BDH Chemicals, Ltd., Poole, Dorset, England. Anhydrous sodium acetate, anhydrous sodium hydroxide, and glacial acetic acid were obtained from Fisons Scientific Apparatus, Loughborough, Leicester, England. All chemicals were analytical grade.

Reagents. *2,6-Dichlorophenolindophenol.* A total of 800 mg of 2,6-dichlorophenolindophenol (sodium salt) was dissolved in about 800 mL of boiling deionized water and filtered into a 1-L volumetric flask. After being cooled, the solution was made up to volume. It was prepared freshly every day and standardized before use.

Metaphosphoric/Acetic Acid (3%/8%). A total of 30 g of metaphosphoric acid was dissolved in 500 mL of deionized water. Glacial acetic acid (80 mL) was added and the mixture made up to 1 L.

Acetate Buffers (0.5 M). The following buffers were used: pH 3.2, 1.23 g of sodium acetate and 30 mL of glacial acetic acid per L; pH 4.5, 16.41 g of sodium acetate and 22 mL of glacial acetic acid per L; pH 6.0, 39.38 g of sodium acetate and 1.6 mL of glacial acetic acid per L.

Copper Sulfate. A total of 3.9395 g of $CuSO_4 \cdot 5H_2O$ was dissolved in 100 mL of deionized water. This solution contained 10 mg of Cu^{2+}/mL. A total of 100 μL diluted to 100 mL with water was used to give a Cu^{2+} concentration of 10 ppm.

Apparatus. A Baskerville bench rocking autoclave unit with pressure vessel assembly (Baskerville & Lindsay, Ltd., Manchester, England) was used for all hydrogenations. An oxidation test for the lubricating oil apparatus (Stanhope-Seta, Ltd., Surrey, England) was used for all oxidations.

Methods. *Preparation of Raney Nickel Catalyst.* A total of 65 g of sodium hydroxide pellets was dissolved in 250 mL of deionized water and cooled to 50 °C in a water bath. A total of 50 g of nickel/aluminum alloy was added very slowly to the flask, keeping the temperature between 48 and 52 °C by addition of ice to the water bath. After all the alloy had been added, the flask was held at 50 °C for 1 h. The catalyst was then washed with water until the supernatant was no longer alkaline to litmus. After filtration, the Raney nickel was stored under water in a refrigerator until required. The catalyst was prepared a day before each hydrogenation. Each batch was assumed to have the same activity.

Preparation of Hydrogenated Glucose Syrups. A total of 450 g of glucose syrup was dissolved in 700 mL of deionized water and transferred to the pressure chamber of the hydrogenator together with one batch of catalyst (i.e., from 50 g of Ni/Al alloy). The temperature was raised to 100 °C and hydrogen admitted to give a pressure of 100 atm. Throughout the reaction, the vessel was agitated and maintained at 100 °C. Hydrogen was admitted at intervals to maintain the pressure at 95–100 atm. This procedure was carried out for 6 h (or longer if hydrogenation was not complete by the time). On completion of the reaction, the contents of the vessel were filtered to remove the catalyst, deionized (Duolite mixed-bed resin), decolorized with 30 g of activated charcoal, concentrated in a rotary evaporator, and then dried under vacuum at 60 °C.

Effect of Carbohydrates on Oxidation Rate of Ascorbic Acid in Aerated Systems. The aim of this experiment was to see whether carbohydrates affect the decomposition of ascorbic acid during autoxidation and/or copper-catalyzed oxidation and whether any changes were affected by pH. Solutions were prepared containing 20% of the chosen carbohydrate, 0.05 M acetate buffer, and 50 mg/100 mL ascorbic acid. The samples were kept at 20 °C, and the Stanhope-Seta fat oxidation apparatus was used to bubble air through them at the rate of 15 L/h.

Aliquots (5-mL duplicates) were removed at suitable time intervals (depending on the reaction rate) and were stabilized with 0.5 mL of metaphosphoric/acetic acid. The residual ascorbic acid concentration was determined by titration with 2,6-dichlorophenolindophenol and expressed as a percentage of the original ascorbic acid concentration. Hence, a profile of ascorbic acid decomposition was obtained in the presence of each carbohydrate (and for a blank containing no carbohydrate). The procedure was carried out at pH 3.2, 4.5, and 6.0 and in unbuffered solution (approximately pH 3.2). The procedure was repeated in the presence of 10 ppm of Cu^{2+} for each carbohydrate at each pH. The results are presented in the figures. Although this procedure would in fact determine all substances formed during the oxidation that react with 2,6-dichlorophenol indophenol, no natural reductones were present. All tests were done in duplicate and results presented are means. Although measurements were made at several time intervals, only the 20-min intervals are recorded.

Effect of Carbohydrates on Oxidation Rate of Ascorbic Acid in Unaerated Systems. Since in most food products

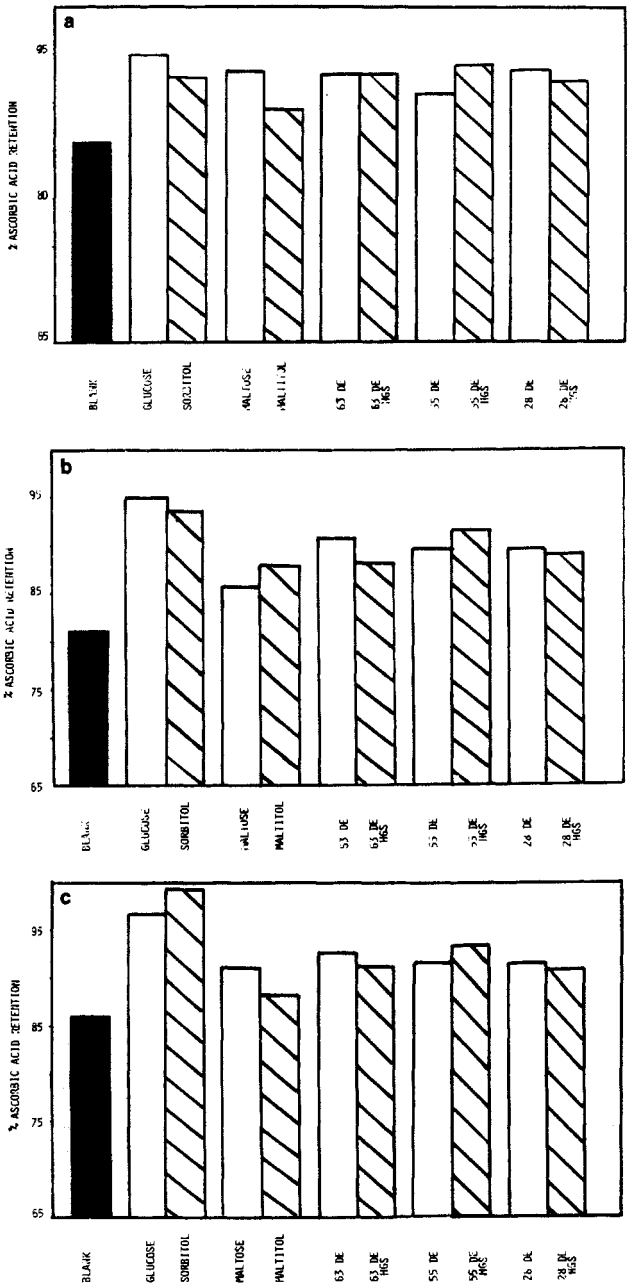


Figure 1. (a) Percentage retentions of ascorbic acid in unaerated solutions buffered at pH 3.2, containing 40% carbohydrates (DE's of glucose syrups shown or hydrogenated syrups, HGS). Maintained at 100 °C for 30 min (blank = zero carbohydrate). (b) Percentage retentions of ascorbic acid in unaerated conditions buffered at pH 4.5, containing 40% carbohydrates [see (a)]. Maintained at 100 °C for 30 min. (c) Percentage retentions of ascorbic acid in unaerated solutions buffered at pH 6.0, containing 40% carbohydrates [see (a)]. Maintained at 100 °C for 30 min.

the oxygen supply is limited, the above experiment was repeated in stoppered flasks. A total of 5 mL of liquid paraffin was floated on top of each sample to prevent dissolution of oxygen when the flasks were opened for sampling. The rate of decomposition was too slow to measure under these conditions, so the temperature was raised to 100 °C in order to speed up the reaction. All other experimental details were the same, except that 12-mL aliquots were withdrawn rather than 5 mL. These were cooled for 5 min in an iced bath and then duplicate 5-mL samples pipetted from them for titration as before.

A few runs were also carried out at different carbohydrate or copper concentrations. Results are shown in the figures and are means of duplicates. Although measurements were made at several time intervals, only the 30-min intervals are recorded.

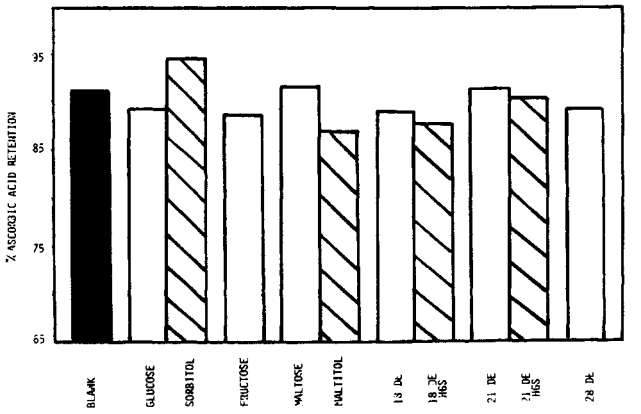


Figure 2. Percentage retentions of ascorbic acid in unaerated, unbuffered solutions, containing 40% carbohydrates (see Figure 1a). Maintained at 100 °C for 30 min.

RESULTS AND DISCUSSION

Figure 1 illustrates the L-xyloascorbic acid percentage retention at three different pH levels and three different concentrations of each carbohydrate without aeration. In this experiment no attempt was made to aerate the samples, nor was any copper catalyst added. Oxidation was achieved at 100 °C. Figure 1 clearly suggests that all carbohydrates confer protection on the vitamin during the early stages of vitamin destruction. Differences in pH do not seem to influence the result, nor is there any general difference between hydrogenated and unhydrogenated types. However, there was some evidence that carbohydrates may actually damage vitamin C on prolonged slow oxidation, particularly with glucose at pH 6.0. This is especially noticeable as the D-glucose concentration is increased and may reflect thermal degradation attributable to the carbonyl function (data available but not shown). It is also possible that the mineral components of reducing sugars aid the destruction of vitamin C. Figure 2 shows the percentage retentions of L-xyloascorbic acid in the absence of buffer. There is no noticeable difference in retention between any of the systems, which indicates that carbohydrates are unable to protect vitamin C per se. The results in Figure 1 therefore presumably arise from the effects of the carbohydrate on the buffer minerals and suggest that the rapid catalytic oxidation of the vitamin would be affected by the presence of carbohydrate.

Figure 3 illustrates the percentage retentions of L-xyloascorbic acid at 100 °C catalyzed by 10 ppm of copper, again without aeration. Oxidation is faster in the presence of copper, and the protective effect of all carbohydrates and their derivatives is clear at this early stage of vitamin destruction. The difference between hydrogenated and unhydrogenated sugars is not easy to interpret, and there is obviously little if any difference between them. The overall result may be complicated by the restricted availability of oxygen and the combinations of solution properties involved. For example, the lower DE syrups have higher viscosities that hinder the mobility of dissolved oxygen, but on the other hand, lower DE systems may be better chelating agents. The same general protection by all carbohydrates is again noticeable in Figure 4, which illustrates the results of catalysis at 100 °C (no aeration) with 50 ppm of copper. Differences between any of the sugar systems are generally absent, though percentage retentions of vitamin are generally lower due to greater concentrations of copper. Percentage protection increases with pH. Figure 5 shows the oxidation L-xyloascorbic acid

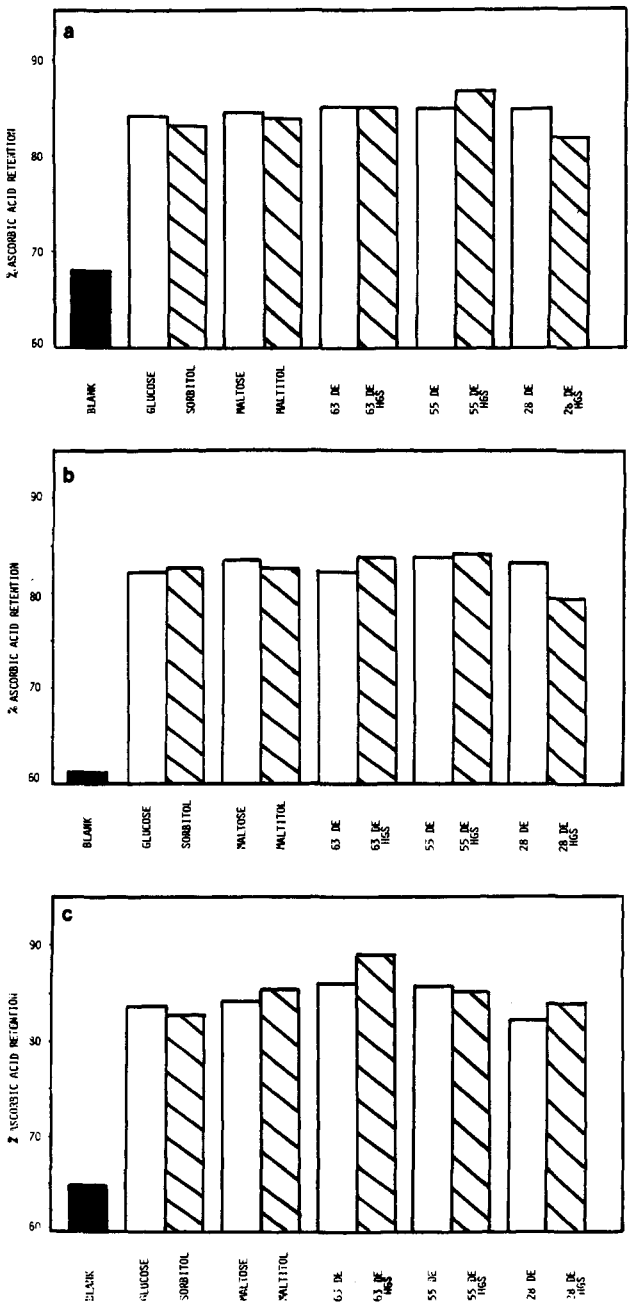


Figure 3. (a) Percentage retentions of ascorbic acid in unaerated solutions buffered at pH 3.2, containing 40% carbohydrates (see Figure 1a) and 10 ppm of Cu^{2+}. Maintained at 100 °C for 30 min. (b) Percentage retentions of ascorbic acid in unaerated solutions buffered at pH 4.5, containing 40% carbohydrates (see Figure 1a) and 10 ppm of Cu^{2+}. Maintained at 100 °C for 30 min. (c) Percentage retentions of ascorbic acid in unaerated solutions buffered at pH 6.0, containing 40% carbohydrates (see Figure 1a) and 10 ppm of Cu^{2+}. Maintained at 100 °C for 30 min.

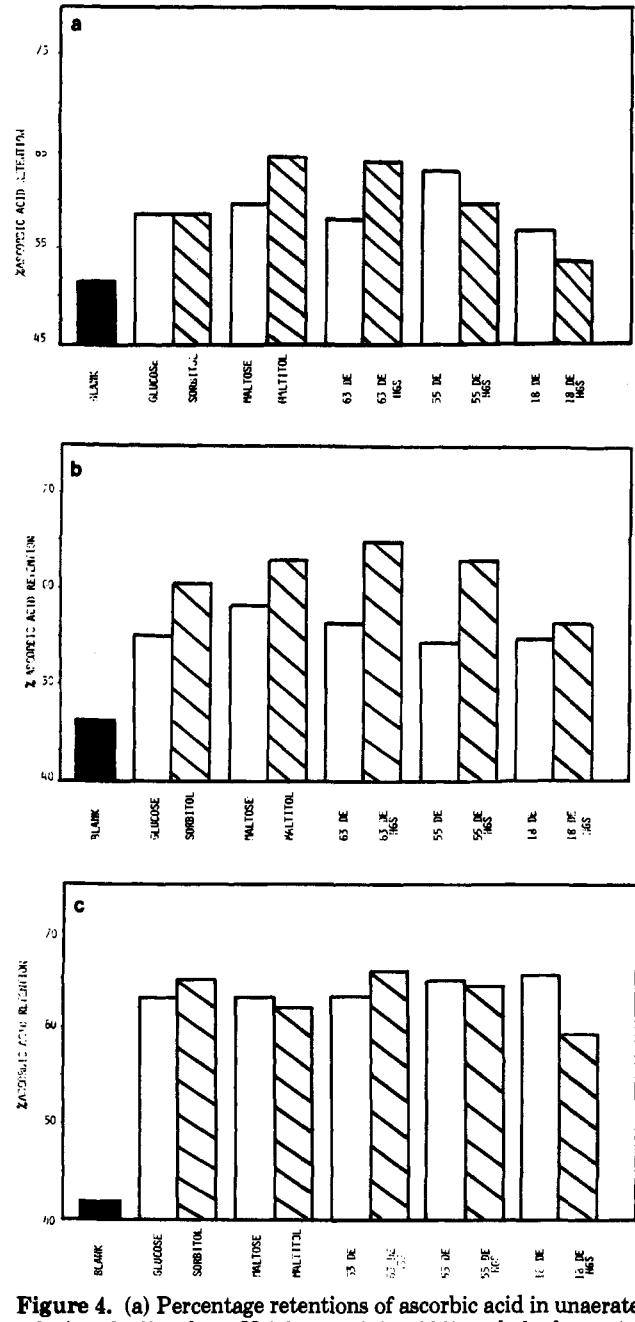


Figure 4. (a) Percentage retentions of ascorbic acid in unaerated solutions buffered at pH 3.2, containing 20% carbohydrates (see Figure 1a) and 50 ppm of Cu^{2+}. Maintained at 100 °C for 30 min. (b) Percentage retentions of ascorbic acid in unaerated solutions buffered at pH 4.5, containing 20% carbohydrates (see Figure 1a) and 50 ppm of Cu^{2+}. Maintained at 100 °C for 30 min. (c) Percentage retentions of ascorbic acid in unaerated solutions buffered at pH 6.0, containing 20% carbohydrates (see Figure 1a) and 50 ppm of Cu^{2+}. Maintained at 100 °C for 30 min.

in an aerated system in the presence of 10 ppm of copper, at 20 °C. Under these conditions oxidation is sufficiently rapid for fine differences between conditions to be made clear, and destruction of the vitamin is accelerated, as anticipated with an increase in pH.

Figure 6 shows the results of the same experiment at the three pH levels in the presence of 20% carbohydrate or the hydrogenated counterpart. All show marked protecting power but differences between them are small. However, a consistent pattern of decreased retention of the vitamin with an increase in pH occurs throughout except with the 55DE glucose syrup. There seems to be no reason for this odd result.

Although all of the results of the experiment lend support to the protective role of carbohydrates in vitamin C solutions, there are no clear differences between the carbohydrates tested. The carbonyl group of free reducing sugars might be expected to stabilize the reducing environment of the L-xyloascorbic acid. However, no differences in protecting power between hydrogenated and unhydrogenated sugars could be demonstrated, and thus the free anomeric centers of these structures appear to be of little significance. Again no differences in protecting power between monomeric sugars and low-DE (largely polymeric) glucose syrups could be established. Thus, physical differences between the solutions were not significant. The one common property of all protective solutions tested was

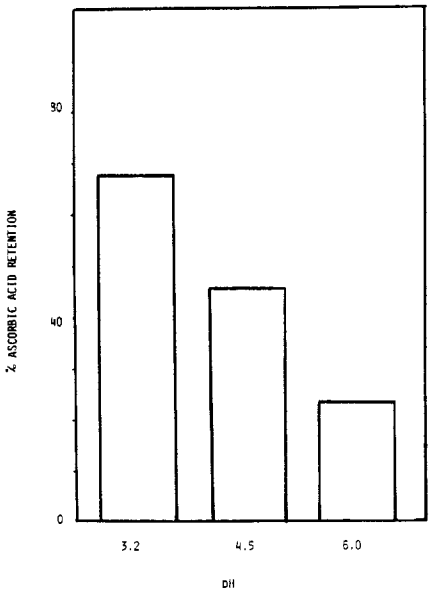
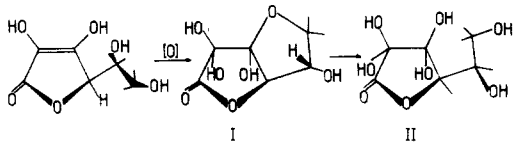


Figure 5. Percentage retentions of ascorbic acid in aerated solutions containing 10 ppm of Cu^{2+}. Maintained at 20 °C for 8 min (no carbohydrate).

Scheme I



their polyhydroxy character, which may in turn have been responsible for their complexing of copper (and buffer minerals) that would otherwise have catalyzed the oxidation. The absence of protection in the absence of copper or buffer (Figure 2) confirms this latter point. The absence of copper in aerated samples gave much less protection than was observed in Figure 6 (results not shown). Once again, however, no differences between carbohydrates were noted. The recent studies of Briggs et al. (1981) have shown that transitional metals, such as copper and iron, complex well with particular carbohydrate and polyol structures. The extent of complexing with copper(II) is particularly great. However, Lewin (1974) has pointed out that dehydroascorbic acid (DHA) also complexes with cations. DHA exists as either the hydrated carbonyl structure II or the hemiacetal structure I (Scheme I) in which a furanose ring is fused to a lactone ring (Hroslef and Pedersen, 1981). Since the rapid loss of DHA will affect the retention of L-xyloascorbic acid, it seems likely that competition for cations between vitamin and polyol structures is of fundamental significance in the intrepretation of our results. ^{13}C NMR studies (Hroslef and Pedersen, 1981) of DHA can all be interpreted in terms of structures I and II and dimerized forms of these. However, the individual complexing power of these structures for cations is not known.

Our results demonstrate that hydrogenation of food carbohydrates does not affect their protective function for vitamin C in oxidizing systems. As hydrogenated glucose syrups possess a similar sweetness to their unhydrogenated counterparts as well as useful physiological advantages (Kearsley et al., 1980, 1982), it seems likely that these stable derivatives may for entirely different reasons be selected for many technological functions. In a variety of food-processing operations, the combined advantages of stability and nonparticipation in browning reactions favors the use of hydrogenated sugars. Their complexing and protective properties are indicative of their potential for nutritionally fortified products.

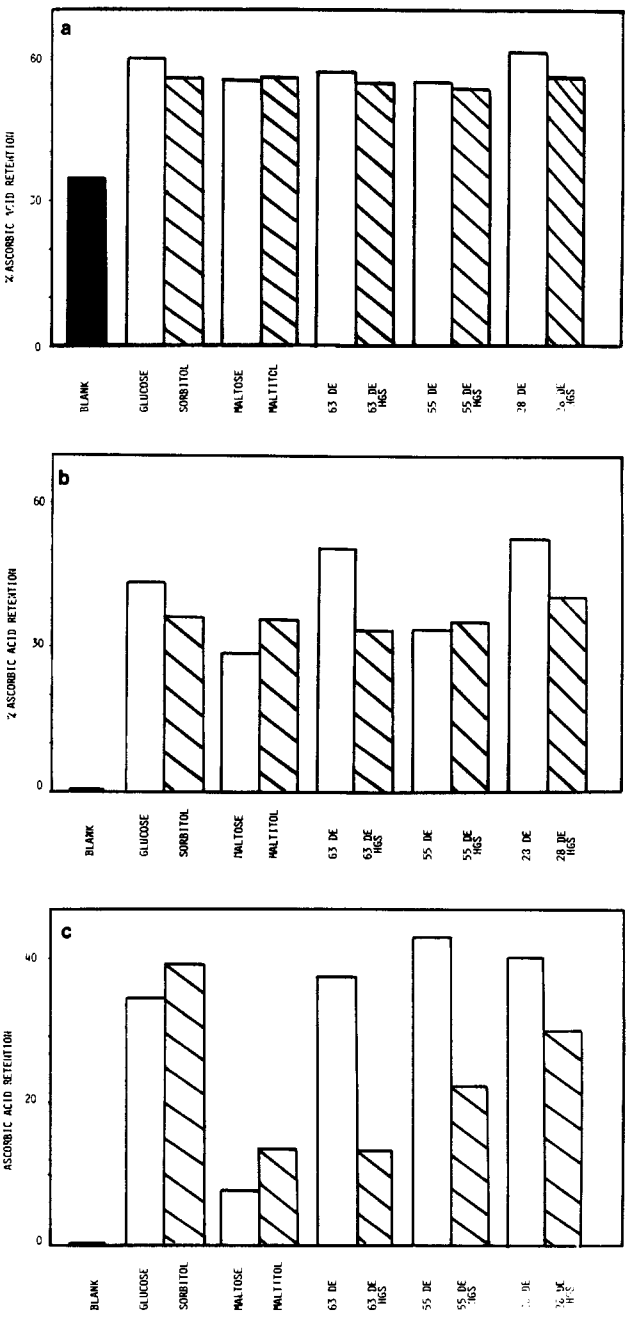


Figure 6. (a) Percentage retentions of ascorbic acid in aerated solutions buffered at pH 3.2, containing 20% carbohydrates (see Figure 1a) and 10 ppm of Cu^{2+}. Maintained at 20 °C for 20 min. (b) Percentage retentions of ascorbic acid in aerated solutions buffered at pH 4.5, containing 20% carbohydrates (see Figure 1a) and 10 ppm of Cu^{2+}. Maintained at 20 °C for 8 min. (c) Percentage retentions of ascorbic acid in aerated solutions buffered at pH 6.0, containing 20% carbohydrates (see Figure 1a) and 10 ppm of Cu^{2+}. Maintained at 20 °C for 8 min.

Registry No. Vitamin C, 50-81-7; D-glucose, 50-99-7; sorbitol, 50-70-4; maltose, 69-79-4; maltitol, 585-88-6; copper, 7440-50-8.

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Determination of Sugars in Yogurt by Gas–Liquid Chromatography

Betty W. Li,* Priscilla J. Schuhmann, and Joanne M. Holden

Individual sugar contents of yogurt sold in the United States were determined by gas–liquid chromatographic analysis of the trimethylsilylated sugar derivatives of freeze-dried samples from 72 composites. Yogurts were obtained from three major grocery stores in each of three cities, Houston, Los Angeles, and Philadelphia, and included 30 brands in three styles of the 10 most popular flavors. Contents of the individual sugars and their sums (total sugar) are reported. Total sugar ranged from 4 to 6% for plain yogurts and from 12 to 18% for flavored yogurts.

Annual per capita consumption of yogurt has risen steadily from 0.25 lb in 1960 to 2.3 lb in 1976 (Kroger, 1978). SAMI (Selling Areas Marketing, Inc.) data indicate an 11% increase in tonnage sales of yogurt in 1982 (*Food Ind. Newsl.*, 1983). The increasing popularity of yogurt among American consumers is reflected in the frequent appearance of new brands and expanded shelf space in supermarkets across the country. This popularity is not surprising in view of the image of yogurt as a convenient, wholesome, high-protein food. Numerous studies, reviewed by Shahani and Chandan (1979) and Deeth and Tamime (1981), have reported the beneficial effects of yogurt in the diet. Recently, Hargrove and Alfrod (1978) and McDonough et al. (1982) found, in controlled studies with rats, that those fed yogurt had increased growth over milk-fed rats.

Carbohydrates, as sugars, are major ingredients in all types of yogurt. Plain yogurt, like milk, contains lactose but also contains galactose produced during fermentation. Flavored yogurts contain other sugars contributed by fruit, fruit preserves, and/or sweeteners. Typically six sugars are found in fruit-flavored yogurts. Relatively few data have been published on the individual sugar contents of yogurts. Southgate et al. (1978) analyzed a small number of plain and flavored yogurts, purchased in Great Britain, using ion-exchange chromatography. Birkhed et al. (1980), in Sweden, included a plain and fruit-flavored yogurt in foods analyzed for sugars and sugar alcohols by gas–liquid chromatography (GLC) but did not report galactose values. Goodenough and Kleyn (1976) used thin-layer chromatography to determine the qualitative and quantitative changes in carbohydrates during the manufacture of unflavored yogurt.

Many analysts consider high-performance liquid chromatography (HPLC) to be the most reliable and rapid method for the determination of individual sugars in foods (Ugrinovits, 1980; Tweeten and Euston, 1980), but the six sugars present in most fruit-flavored yogurts have not been completely separated by any one of the commercially available HPLC columns. Richmond et al. (1982) summarized the current capability for analysis of carbohydrates in dairy products by HPLC. Quantitation of sucrose and lactose is at best uncertain on resin-based columns, and galactose and glucose have not been separated on bonded-phase columns. Barton et al. (1982) have recently reported quantitative separation of glucose and galactose in the neutral sugar hydrolysate of forage cell walls by HPLC. Also, samples of yogurt or other dairy products require considerable cleanup before injection onto either type of HPLC column. We were able to separate and quantitate all the sugars in yogurt in whatever combination they occurred using GLC derivatives (Li and Schuhmann, 1981) prepared from dried samples with no cleanup or extraction. We now report the results of sugar determinations of a nationwide sampling of yogurt including various brands of the most popular flavors.

MATERIALS AND METHODS

Samples. Initial investigation of the market for nonfrozen yogurt indicated that few brands were sold nationwide and that regional brands held a significant share of the market. Because so many brands were available, we prepared composited samples for analysis. We defined three main styles of yogurt on the basis of appearance and label information. In sundae style, plain yogurt is layered above fruit preserves and generally contains active cultures. Swiss style is a stirred product, containing fruit bits and other flavoring throughout. It is usually thickened with gelatin, gums, or modified starch and has been heat-treated after fermentation to inactivate the cultures. A third style, which may be called either blended or French-style yogurt, is a softer gel with uniformly distributed fruit and other flavorings and usually contains active cultures. The sundae and Swiss contain various sweeteners including sugar (Sucrose), corn syrup, honey, fructose, and others. The blended style contains only sucrose as an added sweetener.

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