Preparation and Properties of Sirups Made by Hydrolysis of Lactose

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ABSTRACT

Clear, nearly colorless sirups were prepared from lactose by hydrolysis with either lactase (β-galactosidase) or hydrochloric acid, followed by decolorization, ion exchange demineralization, and concentration. Crystallization of sugars from the sirups was reduced by decreasing total solids from 66 to 60% and the degree of hydrolysis from 95 to 75%; however, overall stability of sirup with respect to both crystallization and mold growth was optimum at 63 to 66% solids and 75% lactose hydrolysis. Stability was increased further by heating sirups to 70 to 75 C and then hermetically sealing the containers. Viscosities were increased by lowering the degree of hydrolysis as well as by increasing total solids. The hydrolyzed-lactose sirups were as sweet as sucrose sirups above 50% total solids but less sweet than sucrose at lower solids. Although increasing lactose hydrolysis from 75 to 95% increased sweetness, differences were small. The sirups possessed good humectant properties at 40% relative humidity.

INTRODUCTION

Spiralling and uncertain sugar prices within the last few years have stimulated research in sugar substitutes. High fructose corn sirup (12) is an example of a sugar substitute that is finding application in food products (5). Lactose, which makes up 66 to 75% of the solids of cheese wheys, is restricted in its applications both by its limited solubility and low sweetness (13, 14). These qualities are improved considerably by lactose hydrolysis (10). Hydrolysis with acid or lactase enzyme produces primarily glucose and galactose and some oliogosaccharides formed by transgalactosidation (1). Our objectives were to prepare highly purified sirups from hydrolyzed lactose and to examine their sweetness, stability, and viscosity for differing hydrolysis and concentrations. Composition of sirups by thin layer chromatography and humectant properties are noted.

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[1] Agricultural Research Service, U.S. Department of Agriculture.

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

MATERIALS

Sugars

Foremost[2] U.S.P. grade lactose was used. Domino brand cane sugar was used for preparing "full sweetness" standards in panel taste testing. For standards in monosaccharide analyses, Bakers AR grade anhydrous glucose, $[\alpha]_D^{25\,C}$ 52.9°, Pfanstiehl anhydrous galactose, $[\alpha]_D^{25\,C}$ 80.2°, Pfanstiehl D-fructose, $[\alpha]_D^{25\,C}$ −92°, and Clinton 100 brand Isomerose were used.

Ion Exchange Resins

Cations were removed by Dowex 50W-X8 20–50 mesh in the H^+ form and anions by Dowex 2-X8 20–50 mesh in the OH^- form.

Lactase

The lactase, "Maxilact" (Enzyme Development Co.), was obtained in two grades, purified (40,000 ONPG U/g, lot #K-903) and crude (20,000 ONPG U/g, lot #K2726).

ANALYTICAL METHODS

Extent of Hydrolysis

The Tauber Kleiner method (11) was used to determine monosaccharides in the presence of lactose. The standard curve was made with a 50/50 mixture of glucose and galactose.

Total Solids

The total solids (TS) of lactose solutions and of the concentrated, hydrolyzed sirups were determined by refractive index (RI) measurements at 27 C. The RI of lactose solutions ranging in TS from 19 to 28% gave a valid percentage TS when the RI was read on the International Scale for sucrose at 20 C. Hydrolysis of the lactose did not change the RI significantly. The TS of the sirups determined by RI agreed with moisture values determined by the toluene distillation.

Viscosity

Viscosity was measured at 25 C with a Brookfield synchrolectric viscometer.

Humectancy

The weight loss of 18.5 to 19.0 g of sirup at 40% RH and 23 C was determined with a uniform-sized sample of sirup in a tared 5.1 cm diameter aluminum dish. After an elapsed time of exactly 15 min, the percent weight loss was computed.

Ash

Ash was determined by the standard method for corn sirup (6) in which 5 g is ashed at 525 C after pretreatment with 3 ml of H_2SO_4 (diluted with 3 vol of water).

Nitrogen

Nitrogen was determined by micro-Kjeldahl (2). The procedure differed only in that the samples were predigested with 3 ml of concentrated H_2SO_4 till foaming stopped, and then Hg0 and K_2SO_4 catalysts were added.

Stability

Sirups were routinely placed in 113 g to 454 g screw cap bottles and sealed. Others were heated to 70 to 75 C for 5 min in loosely capped bottles, and then the tops were sealed. Enzyme processed sirups, hydrolyzed to 95% at 61.4% total solids were adjusted with small amounts of .05 N HC1 or NaOH to pH values in the range 4.0 to 6.8, placed in bottles, and stored at room temperature.

Chromatography

Sirup sugars were separated by thin layer chromatography (TLC) with n-propanol, ethyl acetate, and water (70:20:10) (9). One microliter of a 5% sugar solution or of .2% to .4% lactose was applied to a 250 μ-thick, precoated, 5 × 20 cm Silica gel G plate and developed in a closed tank. The plate was air-dried briefly and then dried 10 to 15 min at 100 C. After being sprayed with 50% H_2SO_4, the plates were developed at 100 C for 20 to 25 min. Records of the spots on the plates were transferred to paper by placing Dietzen 20.5 × 27 cm diazo paper on top of the plates and illuminating from the underside. The spots were intensified as a blue image with NH_3 fumes.

Panel Evaluation

Sirups were evaluated under subdued light for sweetness and other sensory attributes by 12 to 16 trained panel members. Samples were evaluated for sweetness in comparison with standard sucrose solutions by the magnitude estimation procedure. Each judge was supplied with a form indicating number of samples to be tested (with parallel horizontal lines) and limits of sweetness (index mark on each line near left margin indicating no sweetness and another approximately 10 cm to the right indicating the sweetness of a control). Each judge first tasted the control, high-solids sucrose solution which was designated as full sweetness. After rinsing with water between samples, the judges tasted the other concentrations of sucrose and test sirups in randomized order. Each judge made a mark on the lines to the right or left of the full sweetness point as estimates of the sweetness intensity.

Because of sweetness fatigue, a maximum of five samples was judged at one sitting. The judges' responses were quantified by measuring the distance of this mark from the no sweetness point. Sweetness of an unknown sample was computed in terms of the sweetness of the control sucrose by the formula $S = kC^n$, where S = sweetness value obtained as a distance marked on the line, k is constant, C = concentration of sugar as percent, and n = a power exponent (8). The experimentally determined n value for sucrose varied from 1.3 to 2.0 over the concentrations.

PREPARATION OF SIRUPS

Acid Hydrolyzed Lactose

Sirups were prepared by heating in an autoclave either 6 liters of .584 M (18.5% TS) lactose for 70 min or 4 liters of .876 M (26. 8% TS) lactose for 100 min in .1 N HC1 at 121 C. The pH values were 1.25 and 1.00, and the yields of monoses, 89% and 85%. The solutions were decolorized with 1% Norit charcoal, filtered through Celite filter-aid, and passed through a 4.5 × 60 cm column containing 500 ml Dowex 2X-8 in the OH^- form at 40 to 50 ml/min. After adjustment of the pH from 8.7 to 9.0 to 5.5 and 4.8 with 2.5 and 3.6 cc 1 N HC1, the sirups were concentrated in vacuo at 65 to 70 C to 60% total solids (Fig. 1).

Enzyme Hydrolyzed Lactose

A suspension of either .1% purified lactase (wt/vol) or .2% crude lactase (wt/vol) was added to 3 liters of .584 M lactose in distilled water that had been adjusted to pH 6.4 with KH_2PO_4-NaOH buffer (.01 M). After predetermined reaction times at 30 C, the enzyme was inactivated by heating to 75 C. With purified lactase hydrolysis was 75% in 2 h, and with both purified and crude lactases, hydrolysis was over 90% in 6 h. Charcoal (1%) was added to the solutions which then were filtered through filter-aid and passed through Dowex 50W-X8 in the H^+ form. Next, the filtrates at pH 2.2 were passed through Dowex 2-X8 in the OH^- form. The sugar solutions with pH adjusted to 5.4 to 5.6 were evaporated in vacuo at 65 to 70 C to 60 to 66% TS. Sirups with 85% hydrolysis were made by blending 75% and 95% hydrolyzed sirups prepared with purified lactase (Fig. 2).

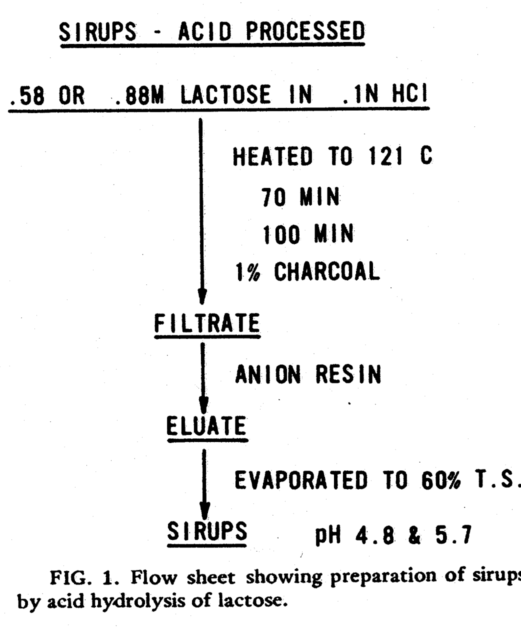

FIG. 1. Flow sheet showing preparation of sirups by acid hydrolysis of lactose.

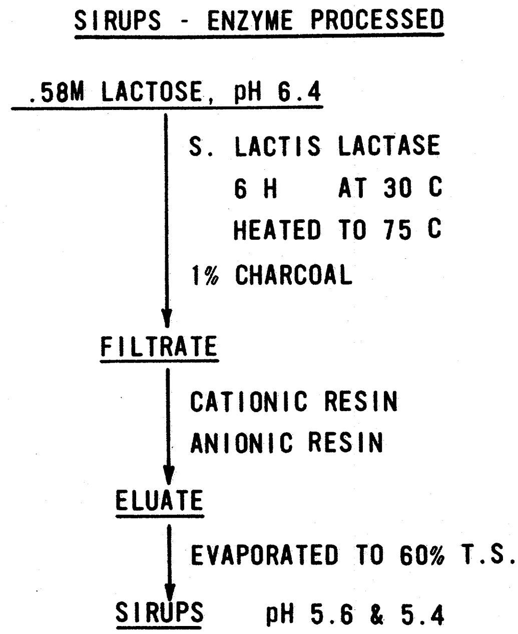

FIG. 2. Flow sheet showing preparation of sirups by enzymatic hydrolysis of lactose.

RESULTS

Properties of Sirups

The effects of the degree of hydrolysis and method of sirup preparation are shown by thin layer chromatograms in Fig. 3 and 4. Samples 3, 4, and 1, in that order (Fig. 3), show a progressive decrease in oligosaccharides with increasing extent of hydrolysis. At 85% hydrolysis or more, the enzyme-prepared sirups show less residual lactose than acid-hydrolyzed sirups (compare, particularly, Sample 4 in Fig. 3 with 4 in Fig. 4). The former also contains minor constituents (oligosaccharides) of better defined R_f values than those of the acid process sirups. Aronson (1) reported that the oligosaccharides formed by enzymic and mineral acid (.3 N H_2SO_4) hydrolysis of lactose differ in configuration, as is suggested by different chromatographic patterns of our

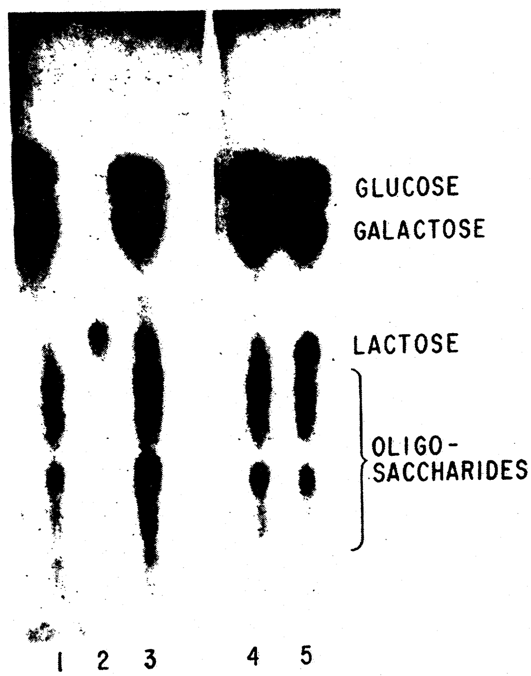

FIG. 3. Thin layer chromatograms of enzyme processed sirups with varying degrees of hydrolysis. 1, 95% of lactose hydrolyzed; 2, pure lactose (.4% solution); 3 and 4, 75% and 85%, respectively, of lactose hydrolyzed; 5, same as 4 plus .4% lactose.

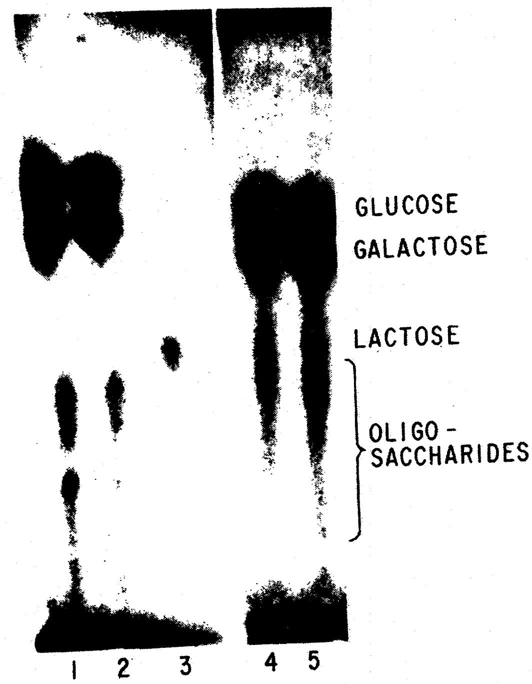

FIG. 4. Thin layer chromatograms of enzyme and acid processed sirups. 1 and 2, 92% and 95%, respectively, of enzyme-hydrolyzed lactose; 3, pure lactose; 4 and 5, 89% and 85% of acid-hydrolyzed lactose.

sirups. Vujicic et al. (15), using 1 to 3 N H_2SO_4, or HC1, detected no oligosaccharides during hydrolysis.

Chromatograms of the enzyme-processd samples do not show evidence of oligosaccharide components with R_f values greater than those of lactose. Wierzbicki and Kosikowski (17), using a different enzyme (β-galactosidase from *Aspergillus niger*) and acid whey as substrate, detected five oligosaccharide components, two of which had R_f values greater than that of lactose. Using lactase from *Saccharomyces fragilis* in 7% to 15% lactose solutions, Aronson (1) detected four oligosaccharides, all having R_f values below that of lactose.

I11-defined zones in the acid-hydrolyzed preparations may indicate a larger number of inseparable components (Fig. 4). Galactose, of R_f .53, and glucose, R_f .59, constitute the bulk of the sugars in all sirups.

TABLE 1. Crystallization rates of unheated sirups made from lactose.

% Enzymatic hydrolysis	Days to crystallization at 23 C (total solids, %)		
	60	63	66
95	7	2	2
85	>40[a]	16	4
75	>50[a]	>250	>250

[a]Mold formed in 28 days.

Stability of Stored Sirups

The degree of hydrolysis affects the crystallization rate; the more extensively hydrolyzed sirups have the poorest stability (Tables 1 and 2). This is substantiated by the data in Table 1 which show that the 95% hydrolyzed sirup crystallized fastest irrespective of solids. Crystallization time decreased with increasing solids content. However, sirups of 60% TS, low

TABLE 2. Crystallization rates of 60% total solids sirups made from lactose.

Type of sirup	% Hydrolysis	Weeks to crystallize at 23 C	
		Unheated	Heated to 75 C and sealed
Enzyme-purified	95	1	6–12
Enzyme-crude	92	1	15>47
HCl	89	1–2	8–13
HCl	85	4–6	>48

enough in sugar content to resist crystallization, were microbiologically unstable within a month. Heating of sirups to 75 C and sealing the container prevented microbiological deterioration as well as increased stability toward crystallization (Table 2).

Further experiments showed that pH, within 4.0 to 6.8, had no effect on crystallization rate in unheated sirups. In sirups containing 61.4% TS and 95% of hydrolyzed lactose, crystallization occurred within 5 to 6 days and mold developed within 6 wk. The sirup at pH 6.8 became slightly yellow.

Sweetness

The sweetness of both acid- and enzyme-hydrolyzed sirups increased relative to sucrose as their total solds increased from 10 to 60% (Fig. 5). Above 50% TS, the sweetness of sirups was not significantly different from that of sucrose at equivalent solids. Increasing the hydrolysis from 75% to 95% tended to increase sweetness at all solids (Fig. 6); however, the differences were not statistically significant. At 30% TS the sweetness of the sirups was equivalent to that of sucrose containing 20 to 22% TS. Similar equivalence was reported by Lin and Nickerson (7) for an 80% hydrolyzed sirup at 30% TS.

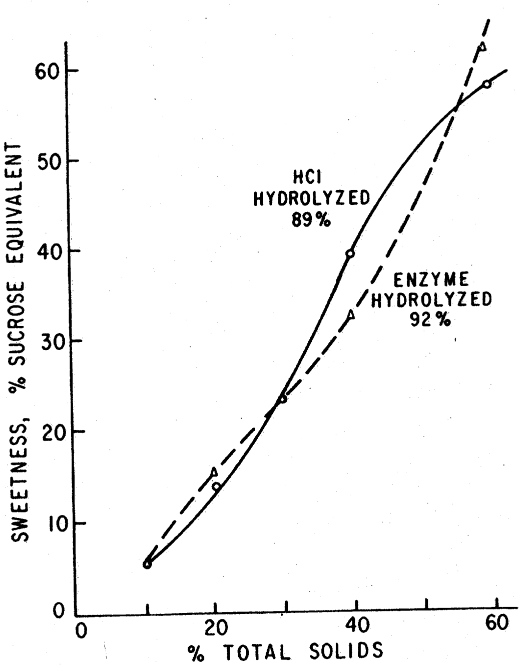

FIG. 5. Effect of total solids of acid and enzyme processed sirups on their sweetness relative to sucrose.

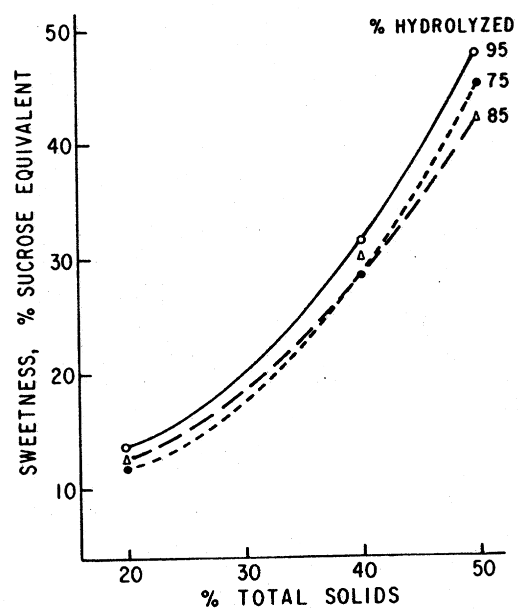

FIG. 6. Effect of extent of hydrolysis of lactose in sirups on their sweetness relative to sucrose.

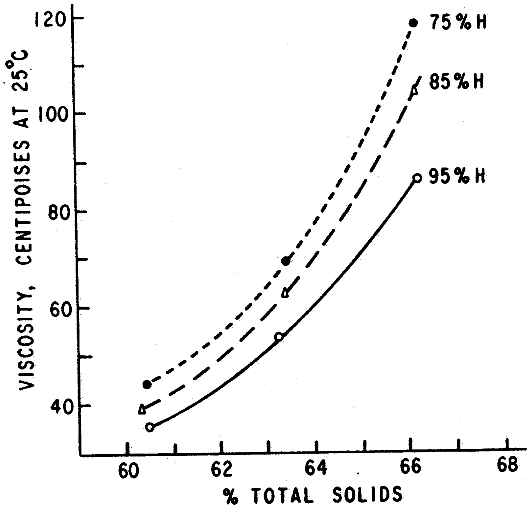

FIG. 7. Effect of total solids and degree of lactose hydrolysis on the viscosity of sirups.

TABLE 3. Loss of moisture from 67% total solids sirups at 40% RH and 23 C in 15 min.[a]

Sirup[b]	% Moisture loss
Sucrose	.156
E–92% H	.139
Invert	.136
Fructose	.133
A–89% H	.132
A–86% H	.131
Isomerose	.131

[a]All significantly different at 5% confidence from sucrose.

[b] E = enzyme hydrolyzed lactose; A = HC1 hydrolyzed lactose.

Viscosity

Increasing the total solids and decreasing the extent of hydrolysis of sirups increased the viscosity of sirups (Fig. 7). The viscosity of the 75% hydrolyzed sirups was below that of sucrose at equivalent solids.

Humectancy

Both enzyme- and acid-hydrolyzed sirups compared favorably with invert sugar, fructose, and isomerose in decreasing the rate of moisture loss at 67% total solids and standardized humidity and temperature (Table 3).

DISCUSSION

To make sirups by enzymatic processing, lactose solutions were buffered with low contents of KH_2PO_4-NaOH at pH 6.4 to accelerate the rate of the reaction. Since corn sirups with low ash are more stable to color change (6), the buffer salts, although low in total amount, were removed by ion exchange. The lactose sirups contained only traces of ash, which was mostly NaCl formed by adjusting the pH of solutions before concentrating them. After hydrolysis, the enzyme was heat-inactivated and largely removed as a precipitate. The sirups were highly purified and had low ash and nitrogen contents (Table 4). The enzyme-prepared sirups had good color stability even when concentrated by boiling and had little if any off-flavors. On extended storage, the acid-prepared sirups turned amber; to many panelists they had bitter after-tastes. Charcoal treatment removed slight objectionable tastes from sirups prepared from crude lactase as well as color from all sirups.

High solids were used in the reaction mixtures to decrease the amount of water to be removed during concentration. To achieve 90% hydrolysis, the higher solids necessitated extension of the reaction time from 2 to 6 h compared with a 5% lactose solution with equal enzyme/g of lactose.

Crystallization occurred more rapidly in

TABLE 4. Ash and nitrogen contents of sirups.

Type of sirup	% Hydrolysis	% Ash	% Nitrogen
Enzyme processed	75	.040	.003
Enzyme processed	95	.015	.002
HC1 processed	85	.047	.000

sirups with 95% hydrolyzed lactose than in those with 75% hydrolysis. An explanation for this must include the relative concentrations and the mutual solubility effects of the several substances, including lactose, galactose, glucose, and oligosaccharides. Some sugar solutions are capable of being highly supersaturated before crystallization occurs. At 75% hydrolysis and 60% total solids, a small degree of supersaturation with respect to galactose, and possibly lactose, may exist; however, crystallization did not occur. Mixtures of closely related organic substances do not crystallize as readily as a single substance (6). Also, high viscosity enhances supersaturation. Figure 7 shows a higher viscosity for 75% hydrolyzed sirups at all solids than those which contained 95% hydrolyzed lactose. In the latter samples, the galactose concentration far exceeded the saturation value for this sugar in an equimolecular mixture of glucose and galactose.

Crystals isolated form 95% hydrolyzed sirups made with lactase consisted primarily of galactose by TLC. This could be expected because the solubility limit of galactose is 32.1% while that of glucose is 50.8% (10).

An explanation for the observation that unheated sirups crystallized more readily than pasteurized samples is conjectural. It is possible that heating dissolved, or otherwise rendered ineffective, any impurities or components of the system having nucleation potential.

No controlled experiments were run on the effect of mechanical shocks on the crystallization rates, but we noted that stirred samples used for viscosity measurements destabilized faster.

The fact that the sweetness of sirups increased relative to sucrose as their total solids increased is predictable in view of the fact that Dahlberg and Penczek (4) reported that the sweetness of glucose is equal to that of sucrose at 40% total solids or higher. Cameron (3) also reported that the relative sweetness of galactose as well as other sugars increased with increasing concentration. He also reported that the sweetness of glucose and galactose are additive, and that glucose is only slightly sweeter than galactose. These findings support our observations that the sweetness of 90% hydrolyzed lactose at 50% total solids or greater is comparable to the same relative concentrations of sucrose.

Sirups from hydrolyzed lactose, because of their sweetness at high solids, might find application in blending with high solids corn or sugar cane sirups, or use in high sugar baked goods. Because of their humectant properties as well as sweetness, they may find application in confections. Less purified sirups (16) isolated from acid whey have been suggested for usage in yogurts, puddings, nutrient fruit juices, and imitation maple sirups.

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