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Isolation of saccharides in dairy and soy products by solid-phase extraction coupled with analysis by ligand-exchange chromatography

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ABSTRACT

The present study reports an improved method to quickly and reproducibly isolate the saccharides from a variety of dairy and soy products utilizing reversed-phase solid-phase extraction to quantitatively remove fats, fatty acids, and lipids followed by desalination and deproteinization by ion-exchange solid-phase extraction with no loss of saccharides during extraction. Analysis of the isolated saccharides was performed by ligand-exchange HPLC. The method presented requires no prolonged heating (thus protecting the saccharides from hydrolysis or isomerization), uses benign reagents, and realizes a significant time savings over existing methods. The isolation and analysis of monosaccharides (glucose, galactose and fructose), disaccharides (lactose and sucrose), and polysaccharides (raffinose and stachyose) from dairy products (whole, reduced fat, and lactose-free milk and yogurt), infant formula (powdered and premixed), and soy beverages were studied in this investigation with recoveries ranging from 88% to 110% in all products studied. We also applied the method to quickly discriminate authentic soy milk from a soy beverage, branded as soy milk.

1. Introduction

The detection and quantitation of mono- and oligosaccharides is of considerable interest in the analysis of dairy and soy products as part of complete quality assurance programs. Dairy and soy are complex mixtures of saccharides, fats, fatty acids, lipids, and proteins, the separation of which makes possible more facile analyses of the individual classes of components. With respect to saccharides, lactose is the main saccharide in dairy products, such as milk and yogurt. Yogurt mixed with fruit may also contain glucose, fructose, and sucrose as well as galactose produced by enzymatic degradation of lactose. The food industry has expended considerable effort to produce formulae for infants and adults similar in composition to human or bovine milk [1]. The major carbohydrates in authentic soy milk are sucrose, raffinose, and stachyose. Because of the economic advantage of soy milk, bovine milk can be extended with soy-milk [2,3] although this may be undesirable due to changes in flavor and carbohydrate and mineral content [4], and such adulteration is prohibited in some countries [3,5,6]. Soy beverages can also be produced from soy protein and one or more sugars and branded as soy milk, although this practice is prohibited in at least one country, namely, Japan [7].

Several procedures are available for saccharide analysis including spectrophotometry [8], mass spectrometry [9], enzymatic analysis [8,10,11], gas chromatography [12–18], electrochemical biosensors [19,20], and high performance liquid chromatography (HPLC) [21–28]. Despite the sensitivity of gas chromatography, sample preparation is laborious and mass spectrometry can be expensive. Few other techniques can rival high performance liquid chromatography for economics, speed, and resolution of sugar mixtures.

Lacking a suitable chromophore for the sensitive detection by UV–visible spectroscopy, saccharide analysis by HPLC is often accompanied by detection with refractive index (RI) [1], evaporative light scattering (ELS) [29], or electrochemical (EC) detection [25]. HPLC coupled with RI detection is economical and offers reasonable sensitivity while ELS detection offers higher sensitivity but can be very sensitive to the matrix, especially when salts are present.

Ligand-exchange chromatography on metal ion-saturated cation exchange media has been shown to be extremely effective for the separation of mono- and oligosaccharides and offers the sugar analyst a reliable technique for the analysis of complex mixtures of sugars and simple organic acids [30–34]. Typical metal ions utilized include Ca^{2+}, Pb^{2+}, and Ag^{+}: each column form producing a particular selectivity for carbohydrates, polyols, or organic acids [32,35]. The use of a cation exchange resin saturated with a metal ion to resolve neutral sugars was first demonstrated in 1960 [36] but it was not until the 1980s that high resolution ligand-exchange stationary phases were commercially available. These substrates made the chromatographic analysis of mono-, di-,

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and trisaccharides relatively simple, using only water as a mobile phase, but requiring elevated temperatures to achieve acceptable resolution and peak bandwidth. The separation mechanism of simple carbohydrates with LEC is described as a combination of ligand-exchange, exclusion, adsorption, partitioning and electrostatic interaction [37–41]. A survey of HPLC column manufacturers shows that modern LEC columns nearly all utilize organic polymer-based supports. The polymer-based support of the ion-exchange media is susceptible to accumulation of proteins from the matrix which may require a suitably acidic solvent (such as 0.01–0.1 M trifluoroacetic acid) to remove [42,43]. The consequence of using an acidic mobile phase on the LEC column is to strip the immobilized cation, thus requiring regeneration after cleaning. In addition, the accumulation of hydrophobic compounds, such as triglycerides (TGs) or fatty acids (FAs), can detrimentally compromise the retention characteristics of the column by coating the column surface or occluding exchange sites [44].

Metal-loaded cation exchange resins for saccharide analysis all suffer from the disadvantage that the injection of samples possessing a non-zero ionic strength will progressively alter the exchange surface thereby changing the selectivity and the resolution of the column [45]. To avoid the effects of ion exchange by adventitious metals in the sample, the mobile phase can be modified with the cation loaded on the column. Indeed, with every manufactured metal-loaded cation exchange column instructions are included for regenerating the column but throughput and repeatability are naturally improved if the interval between obligatory regeneration can be increased. The desalting of samples prior to analysis may thus be preferred for saccharide analysis on metal-loaded ligand-exchange columns.

Most methods of sample preparation of dairy products for saccharide analysis utilize either heat treatment, chemical treatment, or both, to coagulate lipids, TGs, FAs, and proteins. Chemical coagulation of proteins and hydrophobic compounds can be performed under conditions as simple as acidification with oxalic acid [1] or trichloroacetic acid [21] or by the utilization of more complex reagent mixtures, such as the Carrez solutions [3,46,47]. Chemical treatment, however, results in an increase in the ionic strength of the matrix.

Heating is effective in sample preparation and does not affect the ionic strength of the matrix, but in the analysis of oligosaccharides, it can have a detrimental effect due to hydrolysis of the oligosaccharide into the component monosaccharides [16,47]. In addition, it has been shown that during thermal processing lactose can react with the amino group of lysine in the milk casein even at moderate temperatures [48] via the Maillard reaction. The isomerization of lactose to lactulose by the Lobry de Bruyn–Alberda van Ekenstein transformation can also reduce the amount of lactose in the sample when treated with higher temperatures and may cause a more significant loss of lactose [48,49].

An approach to desalting samples with oligosaccharides has been reported in which the oligosaccharides are retained on non-porous graphitized carbon loaded in a solid-phase extraction (SPE) cartridge [50–52]. The packing is preconditioned with, in order, acetonitrile, trifluoroacetic acid, and water after which the sample is applied and the salts and monosaccharides washed off with water. The simple sugars and oligosaccharides are finally eluted with acetonitrile or acid. Although not recommended, the SPE cartridge can be regenerated for reuse. The use of a mixed-bed ion exchange resin has been previously reported but analysis was adversely affected by the co-extraction of neutral sugars. The desalting of sugar extracts has been achieved by batchwise deionization with Amberlite mixed-bed ion exchange media with nearly quantitative recovery [53,54].

We report here an improved method for preparing dairy and soy samples for saccharide analysis by first removing triglycerides (TG), fatty acids (FA), and other nonpolar matrix components by reversed-phase solid-phase extraction followed by simultaneously desalting and deproteinizing with mixed-bed ion exchange solid-phase extraction. The method presented utilizes only water and dilute acetic acid in the sample preparation. We demonstrate the efficiency of the sample preparation and apply it to the isolation and analysis of mono- and oligosaccharides from dairy products (whole, reduced fat, and lactose-free milk, and yogurt), infant formula (powdered and premixed), and soy beverages. We also present the results of our investigation on the ion exchange capacity of the mixed-bed resin, as well as its sugar retention characteristics when used for desalting a solution. The method for sample preparation reported here is an improvement over traditional methods because of reduced sample size and preparation time, no heating steps that may compromise the integrity of the sample through hydrolysis, isomerization, or derivatization of the oligosaccharides, the minimization of hazardous reagents thereby reducing disposal costs, and reduced HPLC column contamination and regeneration frequency.

2. Experimental

2.1. Reagents

All samples of dairy products, soy milk and soy beverages, and infant formula were purchased at local retail stores. Sugar standards, acetic acid, and potassium hydroxide were ACS grade or better and obtained from Fisher Scientific. Solvents for high performance liquid chromatography were HPLC grade (Fisher Scientific). Potassium tartrate tetrahydrate and copper(II) sulfate pentahydrate were obtained from Sigma-Aldrich. Water was deionized by ion exchange to a resistivity $>12\ M\Omega$ cm and filtered to 0.1 μm.

Solid-phase extraction cartridges were purchased from Phenomenex (Torrance, CA). For reversed-phase extractions, 3 mL cartridges packed with 500 mg of Strata C18-E (55 μm, 70 Å) sorbant were utilized. For ion-exchange extractions, 6 mL cartridges packed with 1 g of Strata ABW (55 μm, 70 Å) mixed-bed ion-exchange resin were used.

2.2. Sample preparation

All dairy products were refrigerated at 4 °C until use. Liquid samples (milk, soy milk, and soy beverage) were brought to room temperature and 100 μL aliquots were transferred to 3 mL microcentrifuge vials. Semi-solid samples (e.g., yogurt) were prepared by transferring 100 mg of the foodstuff to a 3 mL microcentrifuge vial. A 1 mL aliquot of 10 mmol/L acetic acid was added to the sample and the mixture was mixed by vortexing. In recovery experiments, a duplicate sample was spiked with the sugars under study. The vial was centrifuged (HBI Microcentrifuge) for 3 min at 26,000*g*. Solid infant formula was prepared as described on the packaging and was otherwise treated identically as liquid samples.

The Strata C18-E solid-phase extraction cartridge was used to extract any remaining triglycerides, fatty acids, and lipids from the supernatant of the centrifuged sample. The Strata C18-E cartridge was preconditioned by flushing with 2 mL of methanol followed by 3 mL of water at a flowrate of 4 mL/min. The supernatant was extracted at a flowrate of 3–4 mL/min and the eluent collected into a polyethylene tube. The sample vial was rinsed with 1 mL of deionized water, which was subsequently used to wash the cartridge bed and combined with the extracted solution.

Salts and proteins were removed from the sample by mixed-bed ion exchange. The Strata ABW mixed-bed ion-exchange cartridge was conditioned by flushing with 2 mL of methanol followed by 3 mL of water at a flowrate of 4 mL/min. The entire

eluent obtained from the reversed-phase extraction was extracted with the ABW cartridge with an additional 1 mL of water to rinse the vial and flush the ABW cartridge. The extract was used for HPLC analysis.

2.3. Validation of extraction efficiency of the Strata C18-E for triglycerides and fatty acids

Hydrolyzed triglyceride (TG) and fatty acid (FA) analysis was performed on non-solid-phase extracted and extracted milk samples using a modification of a method utilized for vegetable oil [55]. A 100 μL aliquot of whole milk was treated with 1 mL of 10 mmol/L acetic acid, centrifuged, and the supernatant extracted with 3 aliquots of 3 mL each of diethyl ether and the extracts combined. An identical sample was prepared, centrifuged, and supernatant treated to reversed-phase solid-phase extraction. The eluent was extracted with diethyl ether in the same manner as the non-solid-phase treated sample. A 100 μL aliquot of milk, treated with acid but not centrifuged, was extracted with diethyl ether as a control.

The ether extract was transferred to a 15 mL round-bottom flask and evaporated under a stream of nitrogen. The residue was suspended in 5 mL of 0.5 mol/L ethanolic KOH and heated at reflux for 1 h. The solution was transferred to a 50 mL polyethylene centrifuge tube, diluted with 25 mL of water, and acidified to pH 5 with sulfuric acid to convert the fatty acids to their neutral form. The fatty acids were extracted with 3 aliquots of 3 mL each of diethyl ether. The combined ether fractions were evaporated under nitrogen and redissolved in 10 mL of HPLC solvent of 99:1 acetonitrile:1% aqueous acetic acid. Samples of hydrolyzed TG and FA were analyzed by HPLC and the identities of residual fatty acids determined by comparison to the chromatogram of the authentic FAs.

2.4. Validation of extraction efficiency of the Strata ABW for removing salts and proteins

The ion-exchange capacity of the ABW cartridge was evaluated by passing multiple 10 mL aliquots of 0.255 mg/mL NaCl (4.36 μeq/mL in Na^+ and Cl^-) across the conditioned ion-exchange resin bed and collecting each 10 mL fraction individually. A total of more than 200 mL of the salt solution was extracted. Each fraction was analyzed by ion chromatography for the presence of either sodium or chloride ion.

To study the efficiency of the ABW cartridge to extract proteins, bovine serum albumin (BSA, Fisher Biotech Grade–Fraction V) was used as a representative protein. Fifteen 1 mL aliquots of 0.5 mg/mL BSA (in 10 mmol/L acetic acid) were extracted with a single ABW cartridge, collecting each eluent in individual polyethylene vials. The protein concentration of each vial was determined with biuret using established protocols [56] and the breakthrough concentration determined.

2.5. Retention study of saccharides on the ABW cartridge

The retention of 17 different saccharides on the ABW ion exchange resin was studied by passing a solution of the sugars through the cartridge and analyzing the eluent. Tested solutions contained one or up to 7 saccharides and the solutions were prepared pairwise either free of salts or containing a mixture of 1.2 mg/mL NaCl and 0.26 mg/mL KCl (0.021 meq/mL Na^+, 0.0035 meq/mL K^+, and 0.0215 meq/mL Cl^-). A 2 mL aliquot of the test solution was drawn through the conditioned ABW cartridge followed by rinsing and dilution to 25 mL. Samples for analysis were prepared in triplicate and analyzed by HPLC.

2.6. HPLC analysis

Analysis of samples containing sugars was performed by ligand-exchange chromatography using a Rezex RCM Monosaccharide column (Ca^{2+}-loaded, 25 cm × 7.8 mm, 8 μm d_p, Phenomenex, Inc.) protected with a SecureGuard® guard cartridge (Phenomenex) coupled to a Spectra Physics SP8800 HPLC pump and Thermo Separations RefractomonitorIV refractive index detector. The column was thermostatted at 85 °C. The pure water mobile phase was helium-degassed continuously during analysis and the flowrate was 0.6 mL/min. The manual injection volume was 20 μL and samples were filtered through a 4 mm diameter 0.2 μm PVDF membrane syringe-tip filter (Millipore) during the injector load step.

Ion-exchange chromatographic separations were performed isocratically using a Thermo Separations P4000 pump equipped with UV1000 UV detector (Thermo Separations). The system was plumbed throughout with PEEK tubing. Mobile phase solvents were helium-degassed continuously during analysis. All samples for analysis were filtered at the manual injector through a 0.2 μm-porosity 4 mm-diameter PVDF membrane syringe-tip filter (Acrodisc). The injection volume was 10 μL and the manual injection loop was rinsed with at least 100 μL of solution.

Ion-exchange chromatography was performed using an indirect detection method adapted from the literature [57–59]. Sodium was determined using a Hamilton PRP-X200 column (150 × 4.1 mm, 10 μm d_p) with a mobile phase of 90% 3 mM copper(II) sulfate:10% methanol at a flowrate of 0.6 mL/min. Detection was by indirect UV at 220 nm and quantitation was by comparison of integrated peak area to a linear calibration plot. Chloride was determined using a Hamilton PRP-X100 column (150 × 4.1 mm, 10 μm d_p) with a mobile phase of 97.5% 4 mM *p*-hydroxybenzoic acid (pH 8.5):2.5% methanol at a flowrate of 2.0 mL/min. The mobile phase was helium-degassed continuously during analysis. Detection was by indirect UV at 310 nm and quantitation was by comparison of integrated peak area to a linear calibration plot.

Fatty acid analysis was performed by reversed-phase chromatography using a Ultrasphere ODS column (25 cm × 4.6 mm, 5 μm d_p, Phenomenex, Inc.) protected with a SecureGuard® guard cartridge (Phenomenex) coupled to a Spectra Physics SP8800 HPLC pump and refractive index detection (Thermo Separations Refractomonitor IV). The mobile phase of 99:1 acetonitrile:1% aqueous acetic acid was helium-degassed continuously during analysis and the flowrate was 1.0 mL/min. The manual injection volume was 20 μL and samples were filtered through a 4 mm diameter 0.2 μm PVDF membrane syringe-tip filter (Millipore) during the injector load step.

3. Results and discussion

3.1. Validation of the extraction efficiency of the Strata C18-E SPE for TG and FA

The extraction efficiency of fatty acids (FAs) and hydrolyzed triglycerides (TGs) utilizing a C18 SPE cartridge was evaluated and compared to a simple heating/coagulation method. Fig. 1 shows the chromatograms of saponified ether extracts of acidified milk after the milk sample was treated to only acidification (Fig. 1a), acidification and centrifugation (Fig. 1b), or extraction of the FA and TG by C-18 SPE (Fig. 1c). A greater than 80% reduction in FA concentration is observed for all of the detected FAs; however, some FAs and, presumably TGs, remain in the supernatant. Acidification of the milk sample and extraction of the supernatant by C-18 SPE after centrifugation yields virtually

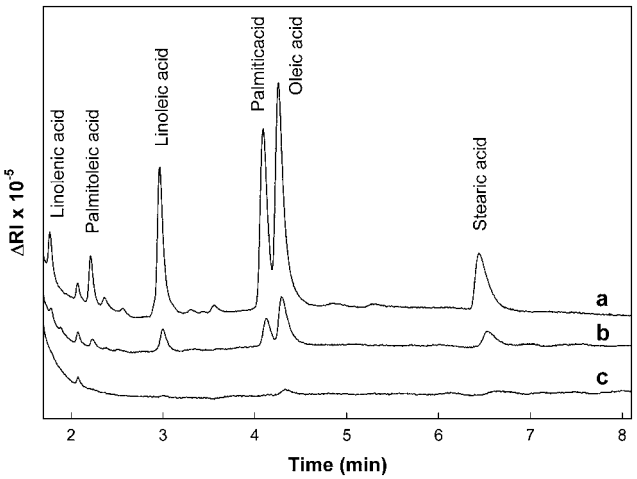

Fig. 1. Natural FAs and FAs produced by saponification of TGs in alcoholic KOH from milk extracts, determined by RP-HPLC. (a) Acidified whole milk, (b) supernatant of acidified whole milk after centrifugation and (c) acidified whole milk after centrifugation and the supernatant polished with C-18 SPE.

quantitative removal of all FAs and FA-producing compounds (Fig. 1c). The identities of the individual FAs in Fig. 1 were determined by comparison to authentic FAs [55].

3.2. Validation of the extraction capacity of the Strata ABW for removing salts and proteins

Fig. 2 shows the breakthrough curves for sodium ion and chloride ion on the Strata ABW mixed-bed ion exchange resin. Ten milliliter aliquots of a solution containing 4.36 μeq/mL Na^+ and Cl^- were sequentially applied to the cartridge. The remaining Na^+ in the eluent was determined by indirect UV detection ion chromatography and plotted against the total amount of Na^+ (in meq) applied to the ion-exchange media. Fig. 2a shows that breakthrough occurs at an extraction capacity of 0.54 meq Na^+/g resin. The measurement of Cl^- was performed at the same time yielding breakthrough at a capacity of 0.52 meq Cl^-/g resin.

Fig. 3 summarizes the results obtained when 15 aliquots of 0.5 mg/mL BSA in 10 mmol/L acetic acid were sequentially applied to an ABW SPE cartridge. Samples were not heated to prevent coagulation of the protein. After each addition, the eluent from the cartridge was analyzed for the presence of protein with biuret [56]. Protein breakthrough occurs at 7 mg of BSA. Prior to the sharp increase in non-retained protein in the eluent, the effectiveness of the ABW cartridge to retain protein is quantitative. Similar results were observed for casein with the inflection at about 3 mg. With a molar mass about one-third that of BSA and possessing a nearly identical isoelectric point, we conclude that the molar capacity of the ABW cartridge for casein is approximately the same as BSA.

3.3. Retention of Saccharides on the ABW cartridge

The retention characteristics of 17 saccharides on the ABW ion exchange resin was studied by passing a solution of individual sugars, or mixtures of sugars, either devoid of salts or containing a mixture of 1.2 mg/mL NaCl and 0.26 mg/mL KCl (0.021 meq/mL Na^+, 0.0035 meq/mL K^+, and 0.0215 meq/mL Cl^-) through the cartridge. The chromatograms in Fig. 4 show a mixture of seven sugars all at a nominal concentration of 1 mM. Fig. 4a is the chromatogram of the control solution containing no salt and not treated to extraction with the ion-exchange cartridge. Fig. 4b is the chromatogram of the control solution after extraction through the ABW cartridge. Fig. 4c shows the chromatogram of the sugar

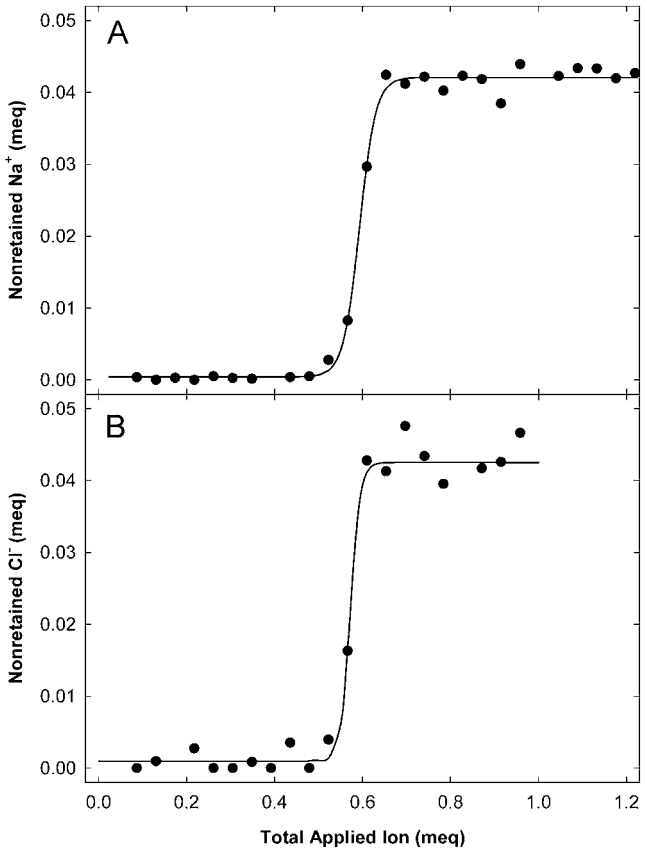

Fig. 2. Breakthrough curves of the amount of (A) non-retained Na+ and (B) non-retained Cl- remaining (in meq) in each 10 mL aliquot containing NaCl after passing through the ABW cartridge plotted versus the total amount of ion (in meq) applied to the ion-exchange cartridge.

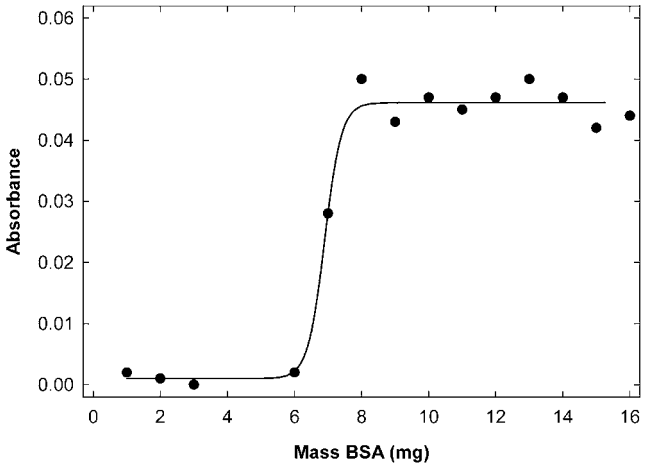

Fig. 3. Breakthrough curve for BSA on the Strata ABW cartridge.

solution identical to the control, but containing salt, after ion extraction with the ion-exchange cartridge. The chromatograms are not statistically different at the 95% confidence interval (CI, Student's-*t*). Fig. 5 shows the results of 17 different saccharides in four classes (sugar alcohols, mono-, di-, and trisaccharides) before passing through an ion-exchange SPE cartridge (control) and after extracting a solution identical to the control (Extract-salt) and extracting a solution identical to the control containing NaCl (Extract+salt). The concentrations of the sugars in the control solution after extraction with the ABW cartridge indicate quantitative recovery of all sugars studied when compared to the

non-extracted control solution. There is no statistical difference indicated at the 95% CI in any recovered sugar concentration between the non-extracted control and extracted control. Likewise, the solution containing the sugars and salts showed quantitative recovery after extraction (Extract+salt in Fig. 5) for all sugars and no statistical differences at the 95% CI are indicated between the Control and Extract+salt solutions after extraction. The Extract+salt solution showed no presence of salt after extraction, as measured by ion chromatography. The recoveries for extraction (without or with salt) range from 94% to 102% and indicate quantitative recovery of each sugar regardless of the ionic strength of the medium (Table 1).

3.4. Analysis of dairy and soy Products

When this method of sample preparation is applied to the analysis of a variety of dairy products and soy beverages, we see nearly quantitative recovery of every saccharide studied (Table 2). Percentage recoveries were determined by comparing unmodified samples and samples spiked with one or more saccharides immediately before starting the preparation sequence. In all cases, recoveries range from 88% to 110% and assays are consistent with reported values for simple mono- and oligosaccharides in each product [60]. Even though complete breakthrough curves were not measured for dairy or soy products, complete extraction of proteins was verified by biuret and for salts by ion chromatography for several authentic samples.

A significant advantage in temporal economics is realized by the sequential SPE steps to remove hydrophobic components (FA, TG, lipids) then simultaneous desalting and deproteinization, without retention of the saccharides. The sample preparation requires 10–15 min, in contrast to 30–60 min, and sometimes more, for other common methods [13,14,21,25,52]. In addition, the potential loss of sample integrity is minimized by eliminating any heating step. Added benefits include a smaller sample size (100 mg or less) and the utilization of only small quantities of reagents (methanol and acetic acid) in the entire sample preparation leading to a lower cost of sampling and waste management.

3.5. Discrimination of authentic soy milk and soy beverage

Soy milk is made from whole dried soybeans which are soaked in water, usually overnight. The beans are then ground with

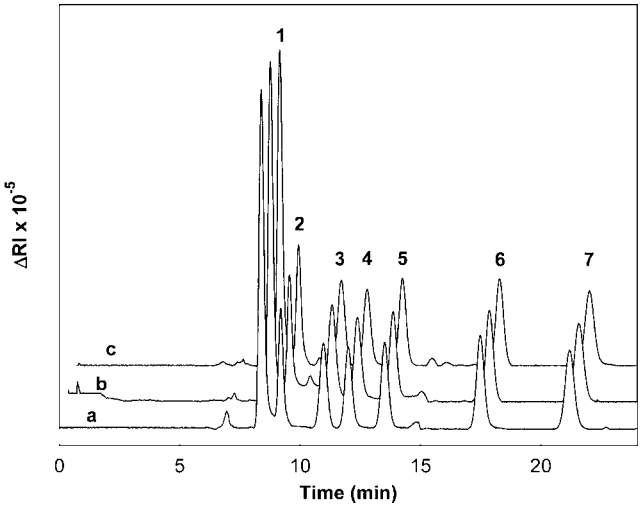

Fig. 4. Ligand-exchange chromatograms of 7 saccharides. (a) control solution, (b) control solution after extraction through ion-exchange SPE cartridge (extract-salt) and (c) solution identical to control containing NaCl and KCl after extraction through ion-exchange SPE cartridge (extract+salt). Peak identities: (1) raffinose, (2) sucrose, (3) glucose, (4) xylose, (5) fructose, (6) mannitol and (7) xylitol.

Table 1
Summary of the average percentage recovery of the sugars studied after extraction of the control solution through an ion-exchange SPE cartridge (Extract-salt), and extraction of a solution identical to control but containing salt (Extract+salt). Values in parentheses are standard deviation in recovery on 3 trials.

	Recovery (%)	
	Extract (− salt)	Extract (+ salt)
Arabinose	97 (1)	99 (0.3)
Mannitol	102 (1)	102 (4)
Sorbitol	100 (2)	100 (3)
Xylitol	102 (3)	97 (3)
Fructose	101 (1)	101 (2)
Fucose	99 (1)	100 (0.3)
Ribose	97 (2)	97 (3)
Mannose	98 (1)	99 (2)
Galactose	99 (2)	95 (5)
Glucose	100 (1)	100 (1)
Xylose	100 (1)	101 (4)
Lactose	95 (1)	96 (1)
Maltose	97 (1)	99 (1)
Sucrose	100 (1)	100 (3)
Maltotriose	99 (1)	99 (1)
Melezitose	100 (1)	99 (3)
Raffinose	97 (2)	94 (4)

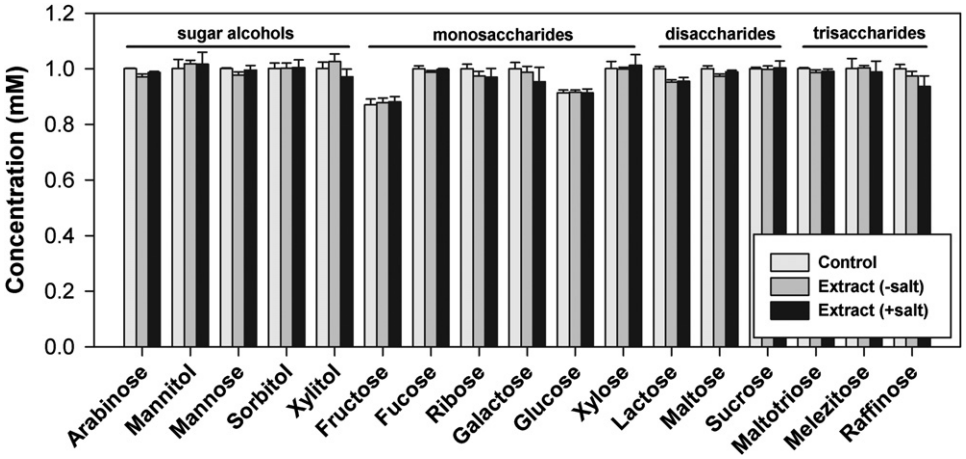

Fig. 5. Summary of the concentrations of 17 saccharides determined chromatographically after no extraction (Control), extraction of the control solution through an ABW cartridge (Extract-salt), and extraction of a solution identical to control but containing salt (Extract+salt).

Table 2
Mean saccharide content and percentage recoveries in dairy and soy products. Values in parentheses are standard deviation in the determination.

Sample	Saccharide	Mean (mg/g)	Recovery (%)
Whole milk	Lactose	47.8 (0.1)	99
2% fat milk	Lactose	48.1 (0.1)	102
Nonfat milk	Lactose	46.9 (0.1)	100
Lactose free	Lactose	3.7 (0.1)	95
	Glucose	21.9 (0.1)	107
	Galactose	20.9 (0.1)	106
Infant formula (liquid)	Lactose	66.8 (0.2)	92
Infant formula (solid) 1	Lactose	55.5 (1.0)	104
Infant formula (solid) 2	Lactose	46.3 (0.8)	90
Infant formula (solid) 3[a]	Sucrose	21.2 (0.3)	96
Yogurt (plain)	Lactose	14.4 (6.7)	88
Yogurt (with fruit)	Lactose	18.0 (0.4)	94
	Sucrose	120.9 (0.3)	93
	Glucose	41.8 (0.4)	91
	Galactose	4.0 (0.1)	93
	Fructose	13.3 (3.7)	89
Soy milk	Raffinose	1.4 (0.2)	90
	Sucrose	8.8 (0.3)	95
	Glucose	1.3 (0.2)	95
	Fructose	1.3 (0.3)	91
Soy beverage	Sucrose	32.9 (0.5)	100

[a] Infant formula 3 labeled "Lactose-free".

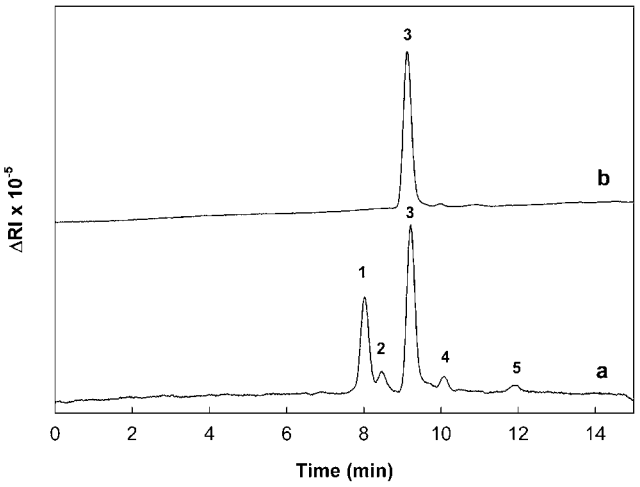

Fig. 6. Chromatograms of (a) authentic soy milk and (b) soy beverage labeled as "Soy Milk." Peak identifications: (1) stachyose, (2) raffinose, (3) sucrose, (4) glucose and (5) fructose. Chromatogram (b) is reduced in scale by a factor of 4.

sufficient additional water to produce the desired consistency and total dissolved solids content in the final product. The resulting purée is brought to a boil to heat-inactivate soybean trypsin inhibitor, improve the flavor, and pasteurize the beverage. Insoluble soy pulp is removed by filtration. In US, "soy milk," "soymilk drink," or "soy beverage" may not be prepared by traditional methods. In fact, many branded soy beverages are simply a stabilized emulsion of oil, soy protein, and sucrose [61].

Utilizing the extraction method reported here, the analysis of authentic soy milk (Fig. 6a) showed a complex saccharide composition consistent with soy milk [3] containing stachyose, raffinose, sucrose, glucose, and fructose. The analysis of the saccharide profile of a soy beverage, branded as "Soy Milk," was discovered to be simply a solution of sucrose with added soy protein (Fig. 6b). With the exception of Japan, there is no widely accepted official "Standard of Identity" for soy beverages [7] so no fraud has been committed. While this may not affect the health benefits derived from the beverage (e.g., for lactose intolerance), if labeling and disclosure requirements in US and other countries become more strict with regard to soy beverages, the solid-phase extraction method to identify a suspect beverage is quick and economical.

4. Conclusion

We have demonstrated an improved method of sample preparation of dairy and soy products for saccharide analysis that is fast, accurate, and reproducible and requires minimal sample volume. The method utilizes only benign reagents and provides sequential polishing of the sample by reversed-phase then mixed-bed ion-exchange solid-phase extraction. Without any heating step, the method insures little risk of chemical modification of the saccharides. The removal of residual hydrophobic contaminants is sometimes ignored [62] but polishing the sample free of hydrophobic and charged compounds protects the ligand-exchange column from contamination and postpones regeneration. This benefit can be extended to other normal and reversed-phase columns, as well.

Using this preparation method, several dairy products were prepared for analysis quickly and giving accurate and reproducible assays. The method was also used to quickly prepare soy beverage samples to investigate their authenticity. Genuine soy milk has a complex panel of saccharides, which was easily distinguished from a soy beverage containing only sucrose. The method can also be applied for the rapid preparation of dairy samples for the detection of adulteration.

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