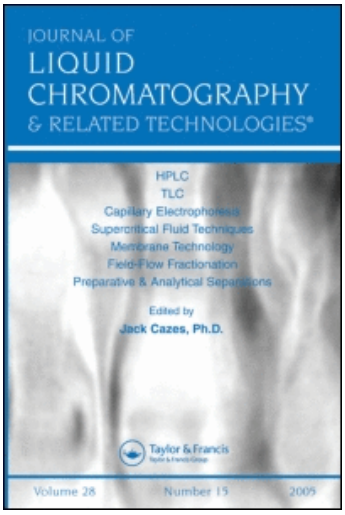

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Rapid Quantitative Method for the Determination of Dextrose, Mannitol, and Sorbitol in Meat Products by Liquid Chromatography

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RAPID QUANTITATIVE METHOD FOR THE DETERMINATION OF DEXTROSE, MANNITOL, AND SORBITOL IN MEAT PRODUCTS BY LIQUID CHROMATOGRAPHY

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ABSTRACT

A liquid chromatographic (LC) method for the determination of dextrose, mannitol, and sorbitol in meat products was developed. Dextrose, mannitol, and sorbitol were extracted from comminuted meat products with 52% ethanol. After filtration, the extracts were purified by passing through a C_{18} Sep-Pak cartridge and two ion exchange resin Econo-Columns in series. After concentration and filtration, extracts were analyzed by liquid chromatography using a cation exchange analytical column and a differential refractometer detector. Homogeneously ground samples of cooked and fresh sausages and ground beef were fortified with dextrose, mannitol, and sorbitol at four different concentrations. Average overall recovery for all three compounds at all four levels of fortification was greater than 80% with coefficients of variation less than 10%.

INTRODUCTION

Sorbitol, a polyhydric alcohol, is permitted in cooked sausages (frankfurters, weiners, knockwurst, etc.) to flavor and to reduce caramelization and charring during the cooking process; dextrose is used to flavor various meat

products. USDA Meat and Poultry Inspection (MPI) regulations limit the quantity of sorbitol in sausages to 2.0 percent, require labeling of the product to show its presence, and prohibit use of sorbitol in combination with corn syrup and/or corn syrup solids. Since dextrose and sorbitol are permissible additives in meat products (sausages), a need for a single, faster, and reliable method for their detection and quantitation is evident. Mannitol, an isomer of sorbitol, is not allowed in meat products. Because of its widespread usage in the food industry, it was considered practical to include mannitol in this procedure, also. Our goal was to develop a simple, rapid, and reliable method for the simultaneous determination of dextrose, mannitol, and sorbitol in meat products.

Dextrose and sorbitol cannot be separated on conventional LC analytical (amino or cyano amino) columns. They either coelute or have poor resolution on carbohydrate columns. Several gas and liquid chromatographic methods have been published for the analyses of dextrose, mannitol, and sorbitol either in mixed standard solutions or in various products: mannitol, sorbitol, and xylitol in chewing gums and confections by LC (1); polyhydric alcohols, arabitol, xylitol, mannitol, sorbitol, and maltitol by LC (2); mannitol and sorbitol in sugarless chewing gum by LC (3); glucose, fructose, glucitol, and mannitol by LC (4); sucrose, glucose, galactose, mannose, talose, fructose, glycerol, and mannitol by LC (5); and sucrose, glucose, fructose, and sorbitol in fruit juices by gas chromatography (6). Most of the preceding methods required either derivatization of sugar/sugar alcohols prior to analysis or LC analytical columns containing pretreated packing materials and use of buffers or aqueous organic mobile phases. The extraction procedures were inadequate to extract dextrose, mannitol, and sorbitol from meat matrices often containing salt, flavorings, citric acid, paprika, monosodium glutamate, antioxidants, and other naturally occurring compounds and/or interferences. Improved column technology enabled us to analyze these sugar and sugar alcohols (glucose, mannitol, and sorbitol) by LC without derivatization and

pretreatment of the packing materials and without using buffers and aqueous organic mobile phases. An LC method using a cation exchange analytical column, water as the mobile phase, and a universal detector (differential refractometer detector) offered the advantages of simplicity, speed, and reliability.

This paper describes an LC method for quantitative determination of dextrose, mannitol, and sorbitol in sausages (cooked and fresh) and ground beef. Recoveries from meat samples fortified with 1.0, 2.0, 3.0, and 4.0 percent of each sugar and sugar alcohol were determined.

EXPERIMENTAL

Apparatus

(a) Magnetic stirrer. - Variable speed, Nuova 7 (Thermolyne Sybron Corp.), or equivalent.

(b) Magnetic bar. - Plastic coated, 1 inch long.

(c) Kohlrausch flask. - 100 ml (Fisher Scientific), or equivalent.

(d) Culture tube with stopper. - 50 ml (VWR 60827-599) or equivalent.

(e) Filter paper. - Folded filter paper 18.5 cm, grade 560 (Schleicher and Schuell, Inc., Keene, NH 03431), or equivalent.

(f) Solvent clarification kit with Durapore filters. - 0.45 um, 27 mm, but without pump and filtering flasks (Waters Associates).

(g) C_{18} Sep-Pak Cartridge. - Waters Associates.

(h) Polypropylene columns. - 0.8 x 4 cm Econo-Column (polypropylene column), holds 2 ml of chromatographic medium and includes an internal 10 ml reservoir (Bio-Rad catalog number 731-1550).

(i) Liquid chromatograph. - Waters Associates, Model 244, with Model R-401 Differential Refractometer Detector, Wisp 710B Sample Processor and Model 730 Data Module, or equivalent, or a Strip Chart Recorder.

(j) Column. - 300 mm x 7.8 mm, 8% crosslinked cation exchange resin column, Aminex HPX-87C (Bio-Rad Laboratories), or equivalent.

(k) Column heater. - Model HPLC Heater (Bio-Rad Laboratories), or equivalent.

(l) Sample filtration apparatus. - Acro LC13 disposable filter assembly with 0.45 micron Fluoropolymer membrane (Gelman Sciences, Inc., 600 South Wagner Road, Ann Arbor, MI 48106), or equivalent.

(m) Limited volume inserts. - Polypropylene insert (250 ul) (Sun Brokers, Inc., P. O. Box 2230, Wilmington, NC 28402), or equivalent.

(n) Ion exchange columns. - Soak resins c(i) and c(ii) separately in distilled water for 1 hour. Rinse with distilled water several times by decantation until supernatants are colorless and pH of supernatants remains constant. Pour cation exchange and anion exchange resins as aqueous slurries onto two separate Econo-Columns (h) until bed heights reach up to 38 mm and 14 mm, respectively. Wash the columns with HPLC water. Position the cation exchange column above the anion exchange column to allow elution in sequence. Do not let columns dry out. The columns are now ready for use.

REAGENTS

(a) Absolute ethanol (200 proof). - U. S. Industrial Chemicals, Inc. (Division of National Distillers Products Corp., New Orleans, LA).

(b) HPLC water. - Prep. using Milli-Q Water Purification System (Millipore Corporation).

(c) Ion exchange resins. - (i) Analytical grade cation exchange resin, AG 50W-X8, 50-100 mesh, hydrogen form (Bio-Rad Laboratories), and (ii) Analytical grade anion exchange resin AG 3-X4A, 20-50 mesh, chloride form (Bio-Rad Laboratories) converted to hydroxyl form by washing with ca 2 volumes of 0.5N NaOH in a column at a flow rate of 1.0 ml per minute. The column was washed with HPLC water until the effluent was chloride free (Test for Cl^- in effluent: Acidify sample with a few drops of concd. HNO_3. Add a few drops of 1% $AgNO_3$ solution. White ppt indicates Cl^-). (To prevent bacterial growth, resins should be stored in a refrigerator or in a cold room).

(d) Mixed standard solutions. - Standard A: 20 mg each of dextrose, mannitol, and sorbitol per ml. - Weigh 10 g of each sugar into a 500 ml volumetric flask. Add ca 400 ml HPLC water, shake vigorously, and hold under running hot water to dissolve. Dilute to volume with HPLC water. Standard B: 15 mg of each dextrose, mannitol, and sorbitol per ml. - Dilute 75 ml of mixed standard A to 100 ml with HPLC water. Standard C: 10 mg each of dextrose, mannitol, and sorbitol per ml. - Dilute 50 ml of mixed standard A to 100 ml with HPLC water. Standard D: 5 mg each of dextrose, mannitol, and sorbitol per ml. - Dilute 25 ml mixed standard A to 100 ml with HPLC water.

(e) HPLC mobile phase. - Filter HPLC water through a Durapore filter into a 2 l filtering flask, and degas under vacuum.

Sample Extraction

Weigh 5.0 g of thoroughly comminuted meat product into a 100 ml Kohlrausch flask. Select a "blank" meat sample as a control and for spiking. Our familiarization protocol requires fortifying with 10 ml of each mixed standard (A, B, C and D) per 5.0 g of samples, representing 4, 3, 2, and 1 percent dextrose, mannitol, and sorbitol, respectively. Add 52 ml ethanol to the sample in the flask. Dilute to volume with HPLC water. Add a magnetic bar, stopper, and stir on a magnetic stirrer for 10 minutes at medium speed. Filter about 45 ml extract into a 50 ml stoppered test tube using prepleated filter paper. (Stopping point. Refrigerate for overnight stopping). Pipet 20 ml filtrate into another 50 ml stoppered test tube. Add 15 ml pet ether, and shake vigorously for 1 minute. Let stand for 5 minutes to separate the layers. Siphon the pet ether layer and discard. Repeat pet ether extraction 2 more times. Pass the extract through a C^{18} Sep-Pak cartridge, collect the effluents in a 250 ml round bottom flask (RBF), and evaporate the extract on a rotary evaporator with a water bath temperature of $40^{0}C$. Dissolve the residue in ca 2 ml HPLC water. Apply to the ion exchange columns, and collect the effluent in a 250 ml RBF. Rinse the flask 4 times with ca 4 ml HPLC water

each time and apply onto the column. Collect all the effluents in the RBF. Add 5 ml ethanol to speed up evaporation. Evaporate the combined effluents on a rotary evaporator at a water bath temperature of 40° C. (Stopping point. Refrigerate for overnight stopping.) Add 2 ml HPLC water and stopper. Swirl on a vortex for 45 sec. and let stand for 1 minute. Filter an aliquat through a 0.45 um Fluoropolymer membrane filter into a limited volume insert and stopper for HPLC analysis.

With each set of samples to be analyzed, process one reagent blank, one control, and one blank sample fortified with mixed standard C. (This represents 2% dextrose, mannitol, and sorbitol.)

Determination

Raise the temperature of the column to 75° C while the solvent is pumped through it. Using isocratic conditions, equilibrate the entire system with mobile phase until a steady baseline is obtained (about 30-45 min.) at a flow rate of 0.6 ml/min and chart speed 1.5 mm/min. Using the Wisp 710B Sample Processor, inject (in duplicate) 25 ul portions of sample extracts and mixed standards. Set range at 32X on Differential Refractometer Detector. Calculate concentration of each sugar in the sample as follows: using peak height and concentration of standards, construct a linear standard curve for each compound based on the formula, $y = mx + b$, where x is concentration (%), y is peak height, m is slope and b is y intercept. The correlation coefficient should be $\geq$ .9900. Calculate recovery of the spiked sample (included with every set) and correct sample results for the running average recovery of the last 10 acceptable recoveries.

RESULTS AND DISCUSSION

Since a universal differential refractometer detector was used to develop this method, the most difficult part of the method was the extraction/cleanup of matrices often containing salt, paprika, citric acid, monosodium glutamate, flavorings, antioxidants, and other naturally occurring compounds and/or inter-

ferences. C_{18} Sep-Pak was not capable of removing all these interferences; however, the C_{18} Sep-Pak was used to filter initial extract, primarily for removing particulates.

Purification step utilizing ion exchange resin Econo-Columns is critical. Various chemical companies supply ion exchange resins. Often, these resins are considered interchangeable or equivalent. Our experience with resin showed that the activity of resin differs from batch to batch even using product from the same company. Prior to application of recovery sample onto ion exchange resin columns, several standards should be eluted to attain approximately 90-95% recovery. Bio-Rad's anion exchange resin AG 3-X4A comes in chloride form which must be converted to hydroxyl form to avoid formation of "inverted sugars" in the extract, if sample contains added sucrose.

The analytical ranges (1. 0 to 4.0%) for all three sugar and sugar alcohols were chosen to cover the regulatory limitation as well as above and below regulatory limitation of sorbitol in the finished product. The separation mode used in this method was ion exchange. Since the method was developed for day to day routine analysis of samples, three objectives were considered in choosing the extraction solvents: (a) extraction solvent should be inexpensive and nontoxic with no unpleasant odor; (b) solvent should eliminate as much soluble protein as possible from the extract; and (c) solvent should extract dextrose, mannitol, and sorbitol from meat matrices. All these requirements were satisfied with 52% aqueous ethanol as extraction solvent. Our ruggedness test indicated that alcohol content in extraction solvent is critical. In addition, sample size of 5 g used in this method is considered representative of the finished product.

LC analysis was performed in approximately 30 min with baseline separation of all three compounds. Figure 1 shows chromatograms of dextrose, mannitol, and sorbitol at the highest and lowest levels of fortification. Under the conditions of the method, the retention times (min) of all three compounds

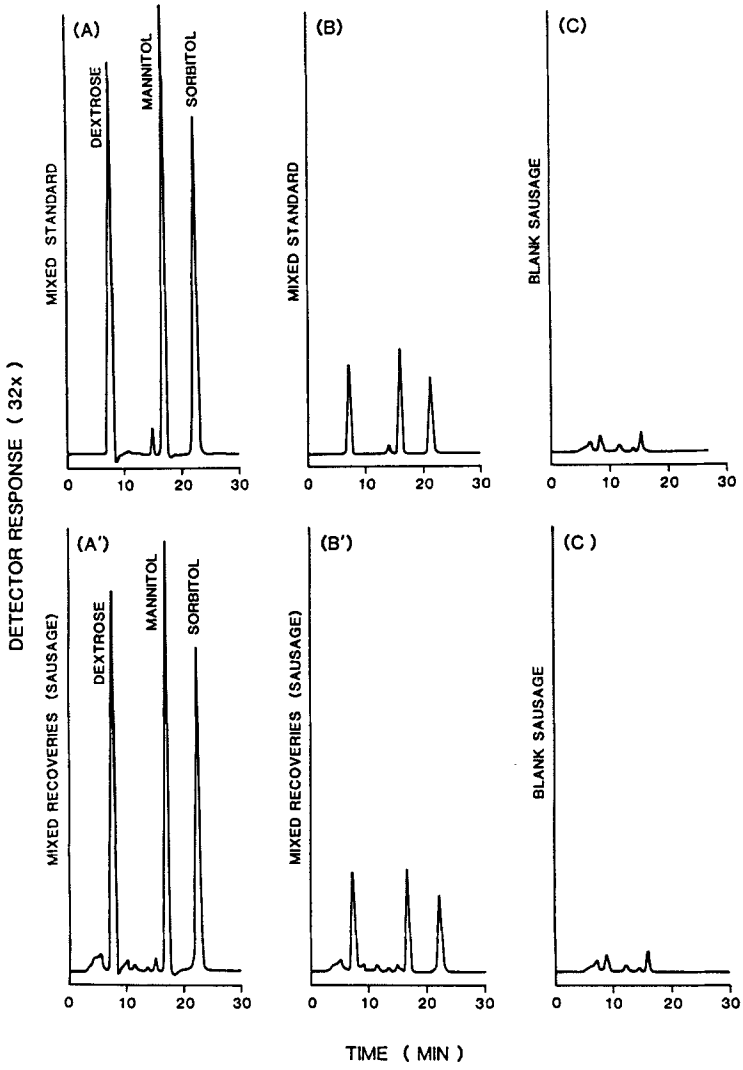

Fig. 1 : LC chromatograms of (A) and (B) mixed standards of dextrose, mannitol, and sorbitol at the highest and lowest levels used in the method and (C) unfortified meat; (A') and (B') mixed recoveries of dextrose, mannitol, and sorbitol at the highest and lowest levels of fortifications used in the method.

Table 1

Statistical data of recoveries for each concentration of dextrose, mannitol, and sorbitol from fresh sausages:

Compound	Concn. %	Number of recoveries	Mean rec. %	SD %	CV %
Dextrose	1.0	12	76.5	6.46	8.44
	2.0	12	72.2	3.98	5.51
	3.0	12	77.3	5.79	7.50
	4.0	12	76.3	5.08	6.66
Mannitol	1.0	12	87.1	6.19	7.10
	2.0	12	82.8	3.01	3.64
	3.0	12	86.6	4.69	5.42
	4.0	12	83.2	4.42	5.31
Sorbitol	1.0	12	88.3	6.33	7.17
	2.0	12	86.3	5.39	6.24
	3.0	12	86.3	2.93	3.53
	4.0	12	83.3	4.58	5.50

were as follows: dextrose, 8.50; mannitol, 17.00; and sorbitol, 22.52. Occasionally a minor interference in the region of dextrose due to the presence of naturally occurring muscle glucose was observed.

Recovery data were based on sausages (fresh and cooked) and ground beef fortified with all three compounds at four different levels. The ranges of concentration used in this method were 1.0 to 4.0 percent for each compound. The average overall recovery for dextrose, mannitol, and sorbitol at all levels of fortification was greater than 80% with coefficients of variations less than 10% as shown in Tables 1, 2, and 3.

The present method is expedient, relatively inexpensive, and sensitive. It is reliable for routine, simultaneous determination of dextrose, mannitol, and sorbitol in meat products and could be applicable to a variety of other products with complex matrices.

Table 2

Statistical data of recoveries for each concentration of dextrose, mannitol, and sorbitol from cooked sausages

Compound	Concn. %	Number of recoveries	Mean rec. %	SD %	CV %
Dextrose	1.0	13	81.9	5.12	6.25
	2.0	13	80.5	5.75	7.15
	3.0	13	82.0	4.02	4.91
	4.0	13	90.2	5.19	5.75
Mannitol	1.0	13	91.3	4.37	4.78
	2.0	13	90.0	5.79	6.37
	3.0	13	90.5	3.37	3.72
	4.0	13	92.8	4.19	4.51
Sorbitol	1.0	13	91.7	4.89	5.33
	2.0	13	90.4	4.86	5.37
	3.0	13	90.5	3.59	3.97
	4.0	13	93.0	4.48	4.82

Table 3

Statistical data of recoveries for each concentration of dextrose, mannitol, and sorbitol from ground beef:

Compound	Concn. %	Number of recoveries	Mean rec. %	SD %	CV %
Dextrose	1.0	12	74.9	7.45	9.95
	2.0	12	71.3	4.71	6.61
	3.0	12	72.8	4.92	6.76
	4.0	12	73.2	4.61	6.30
Mannitol	1.0	12	82.1	6.63	8.07
	2.0	12	83.4	5.05	6.06
	3.0	12	85.8	3.47	4.04
	4.0	12	81.7	3.46	4.24
Sorbitol	1.0	12	84.3	6.72	7.97
	2.0	12	83.5	5.58	6.68
	3.0	12	86.0	3.69	4.29
	4.0	12	82.1	3.73	4.58

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