Ion Chromatographic Determination of Three Fructooligosaccharide Oligomers in Prepared and Preserved Foods

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Fructooligosaccharides (FOS) are short-chain sugars that occur naturally and have dietary benefits for humans. They are widely distributed in nature and are a natural part of the human diet. The objective of this study was to determine the concentrations of 1-kestose (GF_2), nystose (GF_3), and 1^F-β-fructofuranosylnystose (GF_4) in a variety of common processed and prepared foods. An ion chromatographic method was developed for this purpose in which the sugar concentrations were measured using integrated amperometry. The samples were simply prepared by blending with water and filtering the suspensions through a 10000 Da cutoff centrifugal filter. These samples were then injected into the ion chromatograph, which had been programmed for gradient elution, and the areas of the sugar peaks obtained compared to those of standard sugars on a calibration curve. Selected samples were prepared both with and without standard spikes in order to assess the efficiency of the determination. Of the vegetables investigated, artichokes contained by far the most FOS, followed by onions; bananas contained more FOS than other fruits investigated. The method was shown to be simple, convenient, and relatively fast for the quantitation of FOS in processed and prepared food products.

Keywords: *Fructooligosaccharides; food composition; ion exchange chromatography*

INTRODUCTION

Certain prepared or processed foods now found on supermarket shelves, as well as preparations found in health food stores, contain fructooligosaccharides (FOS; Figure 1). They are usually present as either an ingredient (e.g., infant formula) or a natural component (e.g., artichoke hearts). FOS have been found to be distributed as naturally occurring sugars in a variety of plants, fruits, and vegetables (Campbell et al., 1997). Additionally, these materials have been shown to increase the number of bifidobacteria found in the human digestive system (Hidaka et al., 1986; Tomomatsu, 1994; Williams et al., 1994). Wolf et al. (1997) suggested that dietary supplementation with FOS may be beneficial for patients at risk of *Clostridium difficile* infection in long-term care institutions and hospital wards. Because these beneficial sugars have been found and quantitated in fresh foods (Campbell et al., 1997), it is of interest and significance to determine their concentrations in processed and preserved foods for comparison purposes.

For the purposes of this study, FOS have been defined as a combination of the three sugars 1-kestose (1-kestotriose, GF_2; Figure 1a), nystose (1,1-kestotetraose, GF_3; Figure 1b), and 1^F-β-fructofuranosylnystose (1,1,1-kestopentaose, GF_4; Figure 1c). The units of glucose (G) and fructose (F) are in linear chains, always with a single glucose unit at the head, with short chains of fructose units attached. The G−F linkage is (1 → 2)-α, the same as in sucrose (GF), and subsequent F−F linkages are (2 → 1)-β-glycosidic in nature. The three sugars considered in this study, GF_2, GF_3, and GF_4, were determined in various food samples using anion chromatography, a method commonly used for a variety of purposes (Campbell et al., 1997; Prosky, 1999).

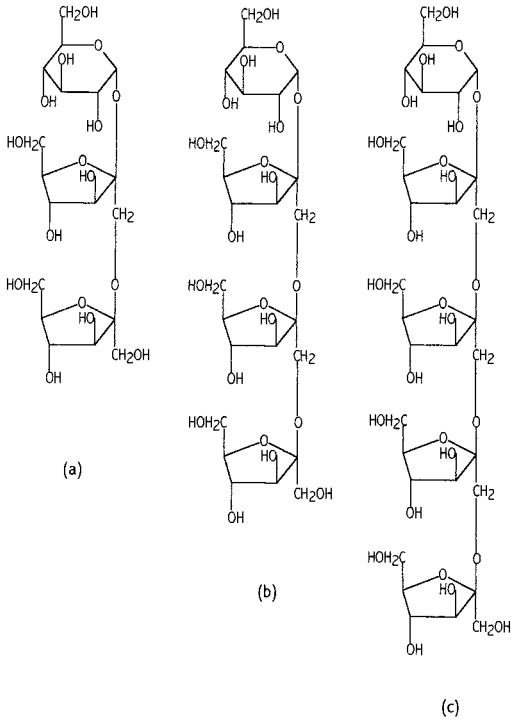

Figure 1. Molecular structures of the FOS investigated: (a) 1-kestose; (b) nystose; (c) 1^F-β-fructofuranosylnystose.

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10.1021/jf000111h CCC: $19.00
Published on Web 09/28/2000

MATERIALS AND METHODS

General Apparatus. Equipment included a Waring blender, or equivalent, with stainless steel 250 mL blending cups; Amicon Centriprep-10 centrifugal filters (P/N 4305; 15 mL capacity, Amicon, Inc. Beverly, MA); a centrifuge capable of holding the filters and spinning them at a top rate of 1100*g*; a source of 18 MΩ deionized water; an apparatus for determining the moisture content of foods; class A volumetric flasks and pipets; general glassware, for example, beakers and conical flasks; a balance capable of measuring to ±1 mg for samples and one capable of measuring to ±0.1 mg for standards; Whatman No. 2v filter paper (12 cm diameter).

Chemicals. All chemicals were obtained as reagent grade or better. As needed chemicals were made by appropriate dilution with 18 MΩ deionized water. Chemical requirements included 200 mM carbonate-free sodium hydroxide, sodium acetate, and 2-propanol. Sugar standards GF_2, GF_3, and GF_4 were obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

Chromatograph. The chromatograph used was a Dionex DX-300 (Dionex, Sunnyvale, CA), equipped with a pulsed electrochemical detector (PED) with working gold electrode operating in the integrated amperometry mode. The column used was a PA1 CarboPac anion-exchange resin column/guard pair (25 cm × 5 mm/5 cm × 5 mm). The eluents (200 mM sodium hydroxide, 100 mM sodium hydroxide + 600 mM sodium acetate, deionized water) were appropriately mixed and delivered to the column using a Dionex GPM programmable pump (AGP-1). The samples were injected (20 μL loop) using a chilled, programmable autosampler set at 10 ± 1 °C. The whole assembly was controlled by a desktop computer using Dionex AI-450 software.

Sample Preparation. The samples investigated, with few exceptions, were obtained from a local grocery store. Liquid samples (e.g., juices and surrounding liquids) were diluted such that the FOS component concentrations would fall within the range of the calibration curve. Typically, 5−10 mL was diluted to 100 mL with deionized water. Denser liquids, such as honey, were measured by weight and again diluted to 100 mL with deionized water. The diluted solutions were centrifugally filtered using Amicon Centriprep 10 10000 Da cutoff filters (1000*g* for 30 min).

Solid samples (e.g., artichokes) packed in liquids were dried using paper towels and weighed directly into a blender cup (typically 5.00−10.00 g ± 10 mg; Waring, 250 mL), along with 50 mL of deionized water. Semisolid samples (e.g., tomato paste) were weighed directly into the blender cup prior to being mixed with water and blending. The samples were blended for 2 min and filtered first through a Whatman No. 2v fluted filter paper (12 cm diameter) and then filtered centrifugally through an Amicon Centriprep 10 filter as described above. The solution obtained was used for the analysis. Dilutions were made of these solutions as needed to fit the range of the standard curve.

Miscellaneous samples (e.g., tablets, flours, and dry cereals) were crushed as needed using a glass mortar and pestle set, weighed directly into a 250 mL blender cup (5.00−10.00 g ± 10 mg), and then diluted with 50 mL of deionized water prior to blending. The solutions were filtered in the same fashion as for the solid and semisolid samples. Again, this solution was used for the analysis and was diluted appropriately if necessary.

Preparation of Standard Solutions. Prior to determinations, ∼5 g quantities of each sugar standard were placed into individual weighing bottles and dried in a vacuum oven (water aspirator) for 12−16 h at 90 ± 2 °C. The still warm powders were then transferred to a vacuum desiccator (water aspirator with trap) for storage. Stock solutions of 1-kestose and nystose (∼10000 ppm) were prepared by weighing 100 ± 3 mg of each into a 10 mL volumetric flask and diluted to the mark with a 7% v/v 2-propanol/water mixture. A stock solution of 1^F-β-fructofuranosylnystose was prepared in the same fashion and stored in a separate 10 mL volumetric flask. The finished solutions were stored in a refrigerator (∼2 °C) until required. When used, the solutions were allowed to warm to room temperature prior to removal of samples for dilution.

Table 1. Pump Program Used for Eluent Mixing and Delivery[a]

time (min)	eluent 1 (%)	eluent 2 (%)	eluent 3 (%)
0.0	50	0	50
2.0	50	0	50
2.1	49	2	49
20.0	42	16	42
25.0	42	16	42
25.1	25	50	25
35.0	25	50	25
35.1	50	0	50
60.0	50	0	50

[a] Eluent 1 = 200 mM sodium hydroxide; eluent 2 = 100 mM sodium hydroxide + 600 mM sodium acetate; eluent 3 = deionized water; flow rate = 1.0 mL/min; injection volume = 20 μL; cycle time (run time) = 60 min.

Table 2. Pulsed Amperometric Cell Conditions

waveform		integration time	
time (s)	potential (V)	begin (s)	end (s)
0.00	+0.40	0.30	0.50
0.50	+0.40		
0.51	+1.20		
0.59	+1.20		
0.60	−0.60		
0.65	−0.60		

Working calibration standards of 1-kestose/nystose were prepared by transferring 2.50 mL of the 1-kestose/nystose stock solution and 1.25 mL of the 1^F-β-fructofuranosylnystose stock solution into a 25 mL volumetric flask and diluting to the mark with deionized water. Of this mixed stock (∼1000 ppm of GF_2/GF_3 and ∼500 ppm of GF_4) aliquots of 0.00, 0.50, 1.00, 1.50, and 2.00 mL were taken and each diluted in a 10.00 mL volumetric flask with deionized water to give approximately 0, 50, 100, 150, and 200 ppm each of GF_2 and GF_3 and 0, 25, 50, 75, and 100 ppm of GF_4.

Chromatographic Procedure. The pump program used for eluent mixing and delivery is shown in Table 1. The gradient selected for these experiments shows a cycle time of 1 h for each injection, thus allowing the column to be cleaned (25.0−35.1 min) and re-equilibrated for the next injection (35.1−60.0 min). The pulsed amperometric detector program used for measuring the carbohydrates is shown in Table 2.

RESULTS

Figure 2a shows a chromatogram of the standards, and an example of a typical sample chromatogram is shown in Figure 2b (it is of the liquid surrounding whole onions). Figure 2b shows, also, the complexity of an apparently simple solution. The GF_2, GF_3, and GF_4 peaks are labeled with their retention times and, in each case, these times matched well with those obtained using a standard solution. As an added precaution, products selected from each group (Table 3A−D) were prepared and then spiked with standard solution, ∼100 ppm of GF_2/GF_3 and ∼50 ppm of GF_4, to aid peak identification as well as to estimate the efficiency (i.e., recovery) of the method (Table 4). Additionally, Figure 2a shows a small peak that occurs after the peak labeled GF_4 has eluted. It was concluded, after additional work had been performed on the GF_4 material, that the small peak was a branched isomer of the molecule shown in Figure 1c. The small peak was used in combination with the larger peak in producing calibration graphs and calculating total GF_4 values in the sample matrices.

The standard sugars produced excellent straight-line calibration graphs ($r \geq 0.99$) throughout the study.

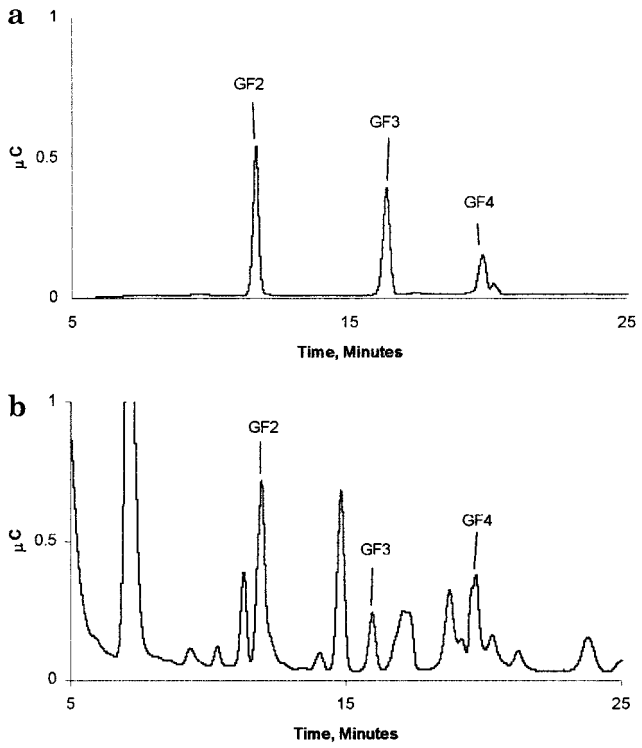

Figure 2. (a) Chromatogram representing a 20 μL injection of a standard FOS solution containing 200 ppm each of 1-kestose (GF_2; 13 min) and nystose (GF_3; 16 min) and 100 ppm of 1^F-β-fructofuranosylnystose (GF_4; 19 min). The small peak occurring after the one labeled GF_4 was later determined to be an isomer of the linear GF_4 molecule shown in Figure 1c. (b) FOS profile of liquid surrounding whole onions (20 μL injection) after extraction: 1-kestose (GF_2; 13 min), nystose (GF_3; 16 min); 1^F-β-fructofuranosylnystose (GF_4; 19 min) (μC = microcoulomb).

Further examination of these graphs allowed a calculation of a practical limit of quantitation for each sugar. This was essentially the same for each of the oligosaccharides at 20 ppm, which translates to 200 μg/g (0.02 wt %) in the solid and liquid samples. Referring to the data tables in which the products studied are divided into groups (Table 3A−D), any sample producing sugar values at or lower than the practical limit of quantitation was quoted as "<0.02%".

Within the group of fruits and fruit products (Table 3A), very few of the products contain major concentrations of FOS. Small amounts (<0.10 g/100 mL) are contained in the apple juices, and progressively smaller amounts are in the other apple products. This is in agreement with conclusions from the work of Campbell et al. (1997). Also, reference to Table 4, the recovery study, shows a similar agreement between the recovery values obtained with these products during this study and those obtained from dried apple matter by Campbell et al. (1997). Other products (e.g., grapes, grape juices, and tomato juice) contain even less FOS than the apple juice. However, one banana puree showed quite a high concentration of FOS (0.13 g/100 g), and one tomato paste showed even more (0.25 g/100 g). Again, these products showed spike recovery values comparable to those obtained by Campbell et al. (1997) for the dry products.

The group of vegetables (Table 3B) showed considerably higher concentrations of FOS among its members than did the fruits and fruit products (Table 3A). For example, the artichoke hearts contained small amounts of FOS (~0.13 g/100 g) whereas the storage liquid surrounding them contained, relatively, large amounts

Table 3. Fructooligosaccharide Composition[a]

product	GF_2[b]	GF_3[c]	GF_4[d]	total[e]
A. Fruit Products				
apples	<0.02	<0.02	<0.02	<0.02
apple juice	0.05	<0.02	<0.02	0.05
apple juice	0.04	<0.02	<0.02	0.04
apple juice	<0.02	<0.02	<0.02	<0.02
applesauce	<0.02	<0.02	<0.02	<0.02
applesauce, stage 1	<0.02	<0.02	<0.02	<0.02
bananas, stage 1	0.11	<0.02	0.02	0.13
bananas, stage 1	0.02	<0.02	0.02	0.04
bananas, stage 1	0.02	<0.02	0.02	0.04
banana chips	<0.02	<0.02	<0.02	<0.02
grapes, seedless	<0.02	<0.02	<0.02	<0.02
grapes, seedless, liquid	<0.02	<0.02	<0.02	<0.02
Concord grape juice, can	<0.02	<0.02	<0.02	<0.02
Concord grape juice, jar	<0.02	<0.02	<0.02	<0.02
Concord grape juice, juice-makers	<0.02	<0.02	<0.02	<0.02
white grape juice	<0.02	<0.02	<0.02	<0.02
tomato juice	<0.02	<0.02	<0.02	<0.02
tomato paste	0.03	<0.02	0.22	0.25
tomato puree	<0.02	<0.02	<0.02	<0.02
tomato sauce	<0.02	<0.02	<0.02	<0.02
B. Vegetable Products				
artichoke hearts	0.04	0.05	0.04	0.13
artichoke hearts, liquid	5.40	6.60	3.10	15.10
artichoke hearts	0.04	0.05	0.04	0.13
artichoke hearts, liquid	5.60	6.00	3.30	14.90
artichoke hearts, marinade	1.80	3.20	2.00	7.00
artichoke hearts	<0.02	<0.02	<0.02	<0.02
asparagus spears	<0.02	<0.02	<0.02	<0.02
asparagus spears, liquid	<0.02	<0.02	<0.02	<0.02
asparagus spears	<0.02	<0.02	<0.02	<0.02
asparagus spears, liquid	<0.02	<0.02	<0.02	<0.02
garlic powder	0.14	0.08	<0.02	0.22
onions, liquid	2.90	2.60	0.63	6.13
onions, whole	0.02	0.02	<0.02	0.04
onions, dry, minced	1.70	1.60	1.10	4.40
onions, dry, minced	1.60	1.40	0.94	3.94
pumpkin	<0.02	<0.02	<0.02	<0.02
squash, stage 1	<0.02	<0.02	<0.02	<0.02
squash, stage 3	<0.02	<0.02	<0.02	<0.02
sweet potatoes	<0.02	<0.02	0.03	0.03
sweet potatoes, syrup	<0.02	0.30	1.40	1.70
sweet potatoes, stage 1	<0.02	<0.02	<0.02	<0.02
yams	<0.02	<0.02	<0.02	<0.02
yams, candied, syrup	<0.02	0.08	0.51	0.59
yams, candied	<0.02	<0.02	<0.02	<0.02
C. Sugars, Grains, and Flours				
barley flour	0.21	0.22	<0.02	0.43
barley, quick cook	0.14	0.05	<0.02	0.19
brown sugar, dark	0.08	<0.02	0.28	0.36
brown sugar, dark	0.06	<0.02	0.06	0.12
honey	0.06	<0.02	<0.02	0.06
honey	<0.02	<0.02	<0.02	<0.02
molasses, blackstrap	0.39	0.04	<0.02	0.43
oatmeal	<0.02	<0.02	<0.02	<0.02
oat bran	<0.02	<0.02	<0.02	<0.02
oat bran cereal	<0.02	<0.02	<0.02	<0.02
rice cereal	<0.02	<0.02	<0.02	<0.02
rye flour	0.50	0.26	0.20	0.96
rye flour, dark	0.58	0.33	0.26	1.17
wheat bran	0.50	0.03	<0.02	0.53
wheat flour	0.15	<0.02	<0.02	0.15
D. Miscellaneous Products				
formula, infant	0.84	0.82	0.12	1.78
Garlic Plus FOS, tablets	12.20	11.70	1.80	25.70
Garliphants	0.43	0.38	0.07	0.88
Sportalyte	<0.02	<0.02	<0.02	<0.02
sweetener, Japanese	40.00	52.00	7.90	99.90

[a] Fructooligosaccharide concentrations for liquids are g/100 mL, whereas those for solids are g/100 g. [b] 1-Kestose. [c] Nystose. [d] 1^F-β-Fructofuranosylnystose. [e] Total FOS = $GF_2 + GF_3 + GF_4$.

Table 4. Fructooligosaccharide Percent Recovery of Various Foods

product	GF_2[a]	GF_3[b]	GF_4[c]
fruits			
apple juice	83	92	73
applesauce	98	99	93
banana, stage 1	125	129	97
banana chips	104	102	93
grapes, seedless	84	100	interference
Concord grape juice, jar	108	116	102
tomato juice	110	105	96
tomato paste	119	114	80
vegetables			
artichoke hearts, liquid	91	108	97
asparagus spears	79	85	116
asparagus liquid	87	82	118
garlic powder	76	75	70
onions, whole	95	96	85
pumpkin	113	92	84
squash	108	97	90
sweet potatoes	103	101	92
yams	interference	85	87
sugars, etc.			
brown sugar, dark	119	107	88
honey	108	107	109
molasses, blackstrap	108	84	114
miscellaneous			
infant formula	81	83	94
Garlic Plus FOS, tablets	98	97	106
Garliphants	102	93	97
rye flour	70	90	83
Sportalyte	112	109	97

[a] 1-Kestose. [b] Nystose. [c] 1^F-β-Fructofuranosylnystose.

of FOS (15 g/100 mL). This suggests that the FOS content of the artichokes is leached quite efficiently into the surrounding liquid, possibly during the partial cooking process that occurs prior to the sealing of the cans. Interestingly, it is this liquid, which contains most of the FOS, that is drained away prior to domestic and restaurant use. The same observation appears to be true for the whole onions and their surrounding liquid, a major portion of the FOS contained in the onions being leached into the surrounding liquid. Minced, dried onion flakes show the presence of large concentrations of FOS (4 g/100 g), the onion only having been subjected to chopping and drying as opposed to a partial cooking process in the presence of liquid. Finally, the sweet potato syrup and candied yam syrup contained quite large concentrations of FOS (1.70 and 0.59 g/100 mL, respectively), again somewhat larger, relatively, than the corresponding solids.

The sugars, grains, and flours (Table 3C) showed the presence of FOS, dark rye flour (1.17 g/100 g), rye flour (0.96 g/100 g), and wheat bran (0.53 g/100 g) topping the list within the group. Comparison of these results to those by Campbell et al. (1997) shows fair agreement: 0.35 g/100 g for wheat bran as compared to 0.53 g/100 g for the current study. Rye grain was investigated (Campbell et al., 1997) giving a total FOS value of 0.38 g/100 g compared to the two rye flour values of this study of 1.17 and 0.96 g/100 g. This suggests that most of the FOS are contained in the part of the rye grain kernel used to produce the flour, the dark flour containing the largest concentration between the two materials. Generally, sugar and sugar products showed lower FOS concentrations than did the flours, the darker sugars having the most: blackstrap molasses (0.43 g/100 g) and dark brown sugar (0.36 g/100 g).

Little comment can be made about the miscellaneous products in Table 3D. All of these are manufactured to contain FOS, the Japanese sweetener powder being made completely from FOS.

For the purposes of checking the efficiency of the method (i.e., spike recovery), some materials were selected from each group described above and spiked with standard FOS solution. Prior to final dilution, solution extracts of the solids were spiked with standard FOS and these, together with the unspiked solution, were injected into the chromatograph. Liquid samples were prepared in spiked and unspiked forms, and these were used for the investigation. The recoveries are detailed for each selected product in Table 4. For the complete set of products the recovery ranges were as follows: GF_2, 70−125%; GF_3, 75−129%; and GF_4, 70−118%. All sugars were very nearly recoverable within the same range. Overall, the recoveries were as follows: GF_2, 99 ± 15%; GF_3, 98 ± 12%; and GF_4, 94 ± 12%.

DISCUSSION

Of the products analyzed in this study, a number would be expected to contain FOS due to their plant origins. For example, onions and garlic both had measurable amounts of FOS. It has been shown (Bacon and Edelman, 1951; Pollard and Amuti, 1981) that vegetables in this plant family (Amaryllidadeae) contain FOS, a fact which has been reconfirmed more recently (Campbell et al., 1997). The results of the present study suggest that the FOS content of these materials is dependent on the storage procedure (i.e., desiccation retains FOS, liquid storage provides a medium for the removal of FOS). Additionally, artichokes (Compositae family), a major commercial source of larger FOS polymers (i.e., GF_n where $n \geq 5$, perhaps up to 60), which contain 5.8 g of FOS/100 g in the fresh form (Campbell et al., 1997), do lose FOS, as shown in this study (∼0.13 g/100 g), to the surrounding liquid, possibly due to the stringencies of the canning procedure, as conjectured before. Fructooligosaccharides are very soluble, and it is not a surprise that they would leach into the surrounding aqueous environment during the canning process.

The flours and grains (e.g., rye flour, Gramineae family) have been shown to contain FOS in their fresh forms prior to milling (Saunders et al., 1975; Nilsson et al., 1986). These data have been confirmed (Campbell et al., 1997), and the current study shows that the total FOS content is dependent on the form of the product (i.e., fresh or processed). For example, the rye grain investigated by Campbell et al. (1997) had a total FOS content of 0.38 g/100 g, whereas the rye flour in this study contains 0.96 g/100 g of FOS. This suggests that most of the FOS present is contained in the kernel, which is the part of the plant milled for flour.

Tomato and tomato products have been found, generally (Tashiro et al., 1992; Campbell et al., 1997), not to contain measurable amounts of FOS. Tashiro et al. (1992) did mention, however, that small, measurable amounts of GF_2 were to be found in tomato paste. This study did confirm that tomato-based juices, sauces, and purees contained no measurable FOS; it also confirmed the presence of a small amount of GF_2 (0.03 g/100 g) as well as modest quantities of GF_4 (0.22 g/100 g) in tomato paste. This difference might be due to differences in the agricultural origins of the tomatoes used in the manufacturing process or in the manufacturing processes used by different manufacturers.

In conclusion, ion exchange chromatography coupled with pulsed electrochemical detection provides a very useful means of both detecting and quantitating FOS in processed and preserved foods. The tables of data document the presence of FOS in processed and preserved foods, as well as the loss of FOS therefrom, possibly as a result of the food-processing conditions.

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Received for review January 26, 2000. Revised manuscript received August 1, 2000. Accepted August 3, 2000.

JF000111H