Selected Fructooligosaccharide (1-Kestose, Nystose, and 1^F-β-Fructofuranosylnystose) Composition of Foods and Feeds

Joy M. Campbell,[†] Laura L. Bauer,[†] George C. Fahey, Jr.,*[,†] A. J. C. L. Hogarth,[‡] Bryan W. Wolf,[‡] and Diane E. Hunter[‡]

Department of Animal Sciences and Division of Nutritional Sciences, University of Illinois, Urbana, Illinois 61801, and Ross Products Division, Abbott Laboratories, Columbus, Ohio 43215

Fructooligosaccharides (FOS) are naturally occurring sugars with potentially beneficial nutritional effects. They are widely distributed throughout the plant kingdom. An ion chromatographic method was developed to rapidly and accurately measure FOS in selected food and feed ingredients ingested by humans and animals. The objective of this study was to determine the 1-kestose (1-kestotriose; GF_2), nystose (1,1-kestotetraose; GF_3), and 1^F-β-fructofuranosylnystose (1,1,1-kestopentaose; GF_4) content of a wide variety of foods and feedstuffs. After extraction with water and appropriate filtration, samples were chromatographed, using an alkaline sodium acetate gradient, through an ion exchange column and guard fitted to a Dionex chromatography unit equipped with a pulsed electrochemical detector. All samples were prepared both with and without spikes of standards to verify recovery and peak identification. Samples of the Compositae family were highest in total FOS followed by *Allium* species of the Amaryllidadeae family. The method provided excellent separation, recovery, and quantification of the GF_n units of FOS. Accurate quantitation of FOS will allow more precise nutritional formulations to be developed with respect to inclusion of this functional food component in human and animal diets.

Keywords: *Fructooligosaccharides; food composition; high-performance liquid chromatography; ion exchange chromatography*

INTRODUCTION

Functional foods have been described as foods that, by virtue of physiologically active food components, provide benefits beyond basic nutrition and may prevent disease or promote health (Functional Foods for Health Program, University of Illinois). A functional ingredient that has received much attention in the scientific literature is fructooligosaccharides (FOS). Several structurally different oligosaccharides have been referred to as FOS such as neosugar (Tokunaga et al., 1986) and oligofructose (Gibson and Roberfroid, 1995; Roberfroid et al., 1993; Van Loo et al., 1995). We have defined FOS as a mixture of 1-kestose (1-kestotriose; GF_2), nystose (1,1-kestotetraose; GF_3), and 1^F-β-fructofuranosylnystose (1,1,1-kestopentaose; GF_4) (Lewis, 1993) oligosaccharides (Figure 1) that consist of short chains of fructose units linked by (2→1)-β-glucosidic bonds and carry a single D-glucosyl unit at the nonreducing end of the chain linked (1→2)-α- as in sucrose. The GF_n units of interest are used as food ingredients (Speigel et al., 1994) in various nutritional foods and are commercially available as analytical standards (Wako Pure Chemical Industries, Ltd., Osaka, Japan). The term FOS will be used here to encompass the above GF_n units. Fructooligosaccharides are synthesized industrially from sucrose through use of transfructosylating enzymes, such as the enzyme β-fructofuranosidase, obtained from *Aspergillus niger* (Hidaka et al., 1988, 1991; Hirayama et al., 1989).

Fructooligosaccharides naturally occur in many plants including banana, onion, wheat, barley, asparagus, and Jerusalem artichokes (Mitsuoka et al., 1987; Spiegel et al., 1994; Tashiro et al., 1992). Fructooligosaccharides have been shown to exhibit beneficial health effects by stimulating the growth of bifidobacteria in the human colon, by suppression of putrefactive pathogens, and by reduction of serum cholesterol concentrations (Gibson and Roberfroid, 1995; Hidaka et al., 1986; Tomomatsu, 1994). Due to their physiochemical properties, sweetening power, and low caloric value, FOS have been added to pastry, confectionery, and dairy products. Their energy value is theoretically lower than that of sucrose (Roberfroid et al., 1993), since the energy value depends on the extent of absorption in the small intestine and fermentation in the colon. The human small intestine has no enzyme to hydrolyze the glycosidic linkages; therefore, FOS are considered to be indigestible in the human small intestine.

Before food-type products are developed with a "new" functional ingredient, several tasks must be completed. First, the safety of the ingredient must be assessed. The safety of FOS has been documented in various studies (Clevenger et al., 1988; Hidaka et al., 1986; Tokunaga et al., 1989). Furthermore, Tokunaga et al. (1986) demonstrated no significant adverse effects occurred in rats with doses up to 1.67 g/day. Second, validated methods must be developed and published to allow documentation of the level of the ingredient in foods and feeds in the final FOS-containing product. Therefore, before or during the development of new products, valid methodology determining FOS must be developed. The objective of this study was to determine the 1-kestose

* Address correspondence to this author at the Department of Animal Sciences, University of Illinois, 132 Animal Sciences Laboratory, 1207 W. Gregory Dr., Urbana, IL 61801 [telephone (217) 333-2361; fax (217) 244-3169; e-mail g-fahey@uiuc.edu].

[†] University of Illinois.

[‡] Ross Products Division.

S0021-8561(97)00087-3 CCC: $14.00

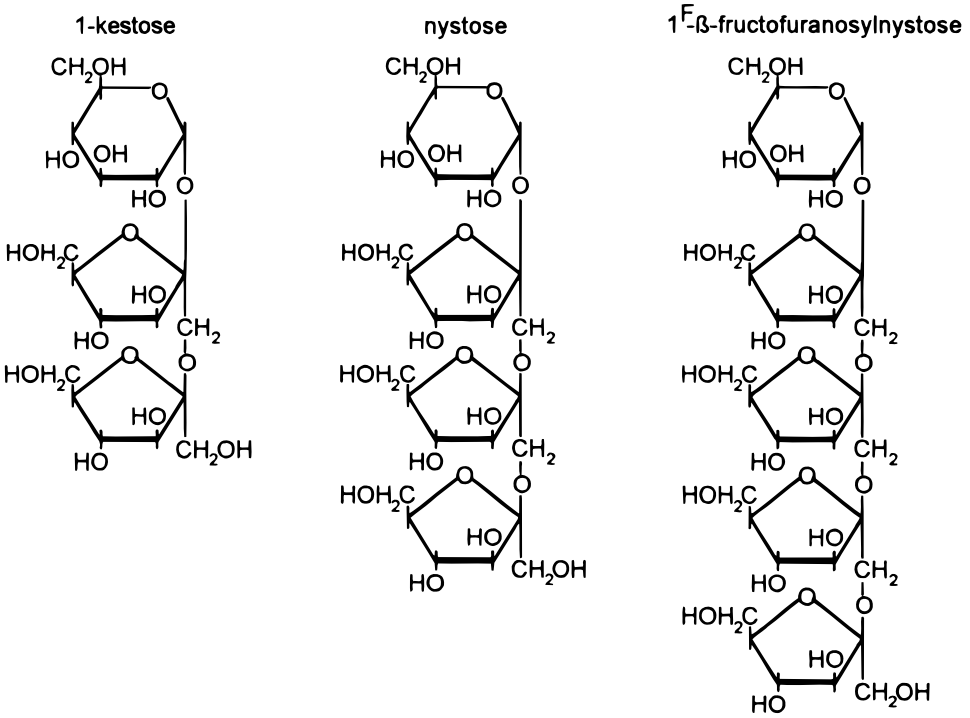

Figure 1. Molecular structures of the fructooligosaccharides that were quantified.

(GF_2), nystose (GF_3), and 1^F-β-fructofuranosylnystose (GF_4) content of a wide variety of foods and feedstuffs.

MATERIALS AND METHODS

Substrates. Food samples were obtained fresh from local retailers. The inedible portion was removed and discarded, while the remaining edible portion was cut into small parts, lyophilized, and ground through a 2 mm screen. Feedstuffs were obtained from local commodity stores and ground through a 2 mm screen. Dry matter was determined according to Association of Official Analytical Chemists methods (AOAC, 1984) to express the FOS content on a dry matter, as well as on an as-is, basis.

Sample Preparation. Approximately 10.00 ± 0.01 g of sample (as is, ground through a 2 mm screen) was weighed and transferred directly into a blender reservoir (Waring blender). An appropriate amount of water (~50 mL or more) was transferred into the blender. Depending upon the sample, sample weight and water volume were adjusted to produce a dilution appropriate for detection within the linear range of the standards (typically 12.5−250 ppm). The mixture was blended for approximately 5 min to produce a homogeneous product. Then the blended mixture was poured into a Büchner funnel containing a Whatman No. 1 filter paper supported in a 25 mL side-arm filter flask. The mixture was filtered to produce a quantity (15 mL) of liquid appropriate for further filtration centrifugally. The centrifugal filter used in this experiment was a 10^4 Da cutoff filter (Amicon, Inc., Beverly, MA). After centrifuging, the filtrate was used for chromatographic analysis.

FOS Separation. FOS content was determined by high-performance liquid chromatography (HPLC). Twenty-five microliters of sample was injected into a Dionex (Sunnyvale, CA) BioLC HPLC fitted with a CarboPac PA1 (4 × 250 mm) analytical column and a CarboPac PA1 (4 × 50 mm) guard column (Dionex Corp., Sunnyvale, CA). The degassed mobile phase consisting of 100 mM NaOH was intially run for 8.0 min. From 8.0 to 30.0 min, the proportion of 100 mM NaOH and 100 mM NaOH/600 mM NaOAc was altered linearly to a final ratio of 88% 100 mM NaOH and 12% 100 mM NaOH/600 mM NaOAc. This concentration was maintained for 6.0 min. After 6.0 min, the eluants were changed to a concentration of 50% 100 mM NaOH and 50% 100 mM NaOH/600 mM NaOAc, which was maintained for 5.0 min to clean the column. The column then was re-equilibrated for 19.0 min with 100 mM NaOH. All eluants were prepared with carbonate-free water and purged with helium. Flow rate was constant at 1.0 mL/min, and elution was conducted at room temperature. Eluted FOS units were detected using a Dionex pulsed electrochemical detector (PED) equipped with a gold working electrode. The PED was operating in the integrated amperometry mode. Total run time per sample was 60.0 min. Appropriate dilutions of a solution containing each of the GF_n units (Wako Pure Chemical Industries, Ltd., Osaka, Japan) were used as the calibration standards. Chromatographic peaks were identified by comparing sample retention times to those of known standard mixtures. Furthermore, to verify peak identity, samples were spiked with a portion of the standard mixture in case of questionable peak identification.

Data Evaluation. To determine linearity of response using the present method, a standard solution of GF_n units, varying from 0 to 300 ppm, was run three times, the average peak areas were calculated and plotted, and regression analysis was performed. On the basis of linear regression, a correlation coefficient of >0.990 was accepted, and the respective linear equations (standard curve) were used for quantification. Components (GF_n units) were quantitated by measuring peak areas and comparing them to a standard curve generated by plotting area counts against concentration of standards (0−250 ppm). Linear regression was used to calculate the calibration curve and the correlation coefficient. Correlation coefficient values were found to be 0.996, 0.998, and 0.999 for GF_2, GF_3, and GF_4 units, respectively. All samples were analyzed in duplicate with and without spike, thus allowing recoveries to be calculated. The duplicate samples were reanalyzed if duplicates differed by >5%. Calculations were based on averaging area counts from analysis of duplicate samples. The average area count was entered into the calibration curve to calculate concentration for the individual GF_n units. Percent recovery was calculated as

$$\% \text{ recovery} = \frac{C_1 - C_2}{\text{spike}} \times 100$$

where C_1 is the concentration of sample with spike, C_2 is the concentration of sample with no spike, and spike is the concentration of spike. Calculation of GF_n unit (mg/g) of FOS content is

$$GF_n \text{ unit of FOS} = \frac{(\text{sample} \times \text{dilution})}{\text{sample weight}}$$

where sample is the GF_n unit concentration of sample without spike (mg/L) from calibration curve, dilution is dilutions (L) used throughout the experiment, and sample weight is the sample weight (g) of as-is sample. An estimate of precision analysis for the different GF_n units was determined for six repeated injections into the HPLC of the same 100 ppm standard solution to calculate a relative standard deviation (RSD) = [standard deviation/mean value] × 100 for repeatability. The practical detection limit for the lower end was determined by six repeated injections of 100, 50, 25, 12.5, 6.25, and 3.125 ppm.

RESULTS

Figure 2 represents a typical chromatogram of a 25 μL injection of a standard solution containing 250 ppm each of GF_2, GF_3, and GF_4. Figure 3 represents the FOS composition of a rye sample after the extraction procedure. The elution profiles were similar with respect to retention times. Peak separation was excellent, allowing all GF_n units to be resolved to baseline, identified, and quantified. Detectability also was very good for the GF_n units. Linear detectability for the PED detector depends upon many factors such as column type, sensitivity settings, and the component being detected. The linearity for the settings and conditions described under Materials and Methods was determined to be 0−250 ppm. The RSD from six injections to determine precision were 0.50, 1.87, and 1.61% for GF_2, GF_3, and GF_4 units, respectively. The RSD for the practical detection lower limit was <2% for the 12.5 ppm sample. The injections determined at 6.25 ppm had >2% RSD, while the 3.125 ppm injections were unable to be integrated with the sensitivity settings of the HPLC. For the conditions and sensitivity settings outlined under Materials and Methods, all GF_n values were in the response range of 12.5−250 ppm. Each component was found to have a practical detection limit of 3−6 ppm.

The FOS composition and percent recoveries for fruits are presented in Tables 1 and 2, respectively. Results are presented as an average on a dry matter (DM) basis and on an as-is basis. The fruits ranged from 0.0 to 10.9 mg of total FOS/g of DM for Thompson grapes and ripe banana, respectively. Greater than 50% of the fruits ranged from 0.2 to 2.0 mg of total FOS/g of DM. Of the fruits analyzed, bananas were highest in total FOS on a DM basis, while grapes and strawberries were lowest. On an as-is basis, the total FOS content ranged from 0.0 to 2.1 mg/g for Thompson grapes and ripe banana, respectively. Again, bananas were highest in total FOS. On an individual GF_n unit basis, the GF_2 unit made up the largest percentage of total FOS, with the GF_4 unit being the second largest. The GF_3 unit was present in the lowest amount for all fruits. The percent recoveries were similar for all fruits, ranging from 83 to 120%. Furthermore, the percent recoveries were similar among the individual GF_n units.

The FOS composition and percent recoveries for vegetables are presented in Tables 3 and 4, respectively. On a DM basis, the Jerusalem artichoke was highest at 286.2 mg of total FOS/g of DM, while several vegetables contained no FOS (ginger root, tomato, and zucchini). Shallot, onion, globe artichoke, raw chicory root, and garlic were moderate in total FOS content, ranging from 10.3 mg/g of DM for garlic to 52.9 mg/g of DM for shallot. On an as-is basis, Jerusalem artichoke

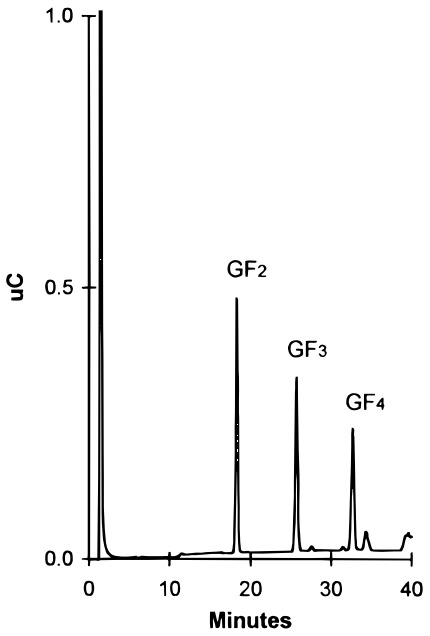

Figure 2. Chromatogram representing a 25 μL injection of a standard fructooligsaccharide solution containing 250 ppm each of 1-kestose (GF_2; 18.35 min), nystose (GF_3; 25.72 min), and 1^F-β-fructofuranosylnystose (GF_4; 32.73 min) (uC = microcoulomb).

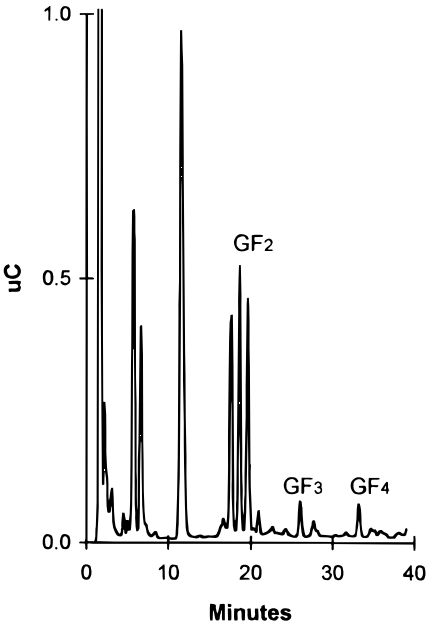

Figure 3. Fructooliogsaccharide profile of rye (25 μL injection) after extraction: 1-kestose (GF_2; 18.70 min), nystose (GF_3; 26.13 min), and 1^F-β-fructofuranosylnystose (GF_4; 33.27 min) (uC = microcoulomb).

was highest in total FOS at 58.4 mg/g as-is, while onion powder was the next highest at 45.0 mg/g as-is. Again, shallot, chicory (raw and roasted), globe artichoke, garlic, and onions were moderate in total FOS content. In contrast to fruits, vegetables were highest in GF_2 units, followed by GF_3 and GF_4 units for most samples. However, acorn squash, celery, Chinese chive, eggplant, endive, Jerusalem artichoke, peas, and radishes were highest in GF_4 units, followed by GF_3 and GF_2 units. Percent recoveries were similar to those of fruits, ranging between 81 and 123% for acorn squash and Roma tomato, respectively. Among the individual GF_n units, percent recoveries were similar.

Table 1. Fructooligosaccharide Composition of Fruits

ingredient	mg/g of DM				mg/g as is			
	GF_2[a]	GF_3[b]	GF_4[c]	total[d]	GF_2	GF_3	GF_4	total
apple, Red Delicious	0.6	0.0	0.0	0.6	0.1	0.0	0.0	0.1
apple, Golden Delicious	0.2	0.0	0.0	0.2	0.0	0.0	0.0	0.0
apple, Granny Smith	0.5	0.0	0.0	0.5	0.1	0.0	0.0	0.1
apple, Jonagold	0.4	0.0	0.0	0.4	0.1	0.0	0.0	0.1
apple, Rome	0.3	0.0	0.0	0.3	0.0	0.0	0.0	0.0
banana	5.9	0.1	0.0	6.0	1.4	0.0	0.0	1.4
banana, green	3.1	0.0	0.0	3.1	0.7	0.0	0.0	0.7
banana, red	1.8	0.0	0.0	1.8	0.5	0.0	0.0	0.5
banana, ripe	8.6	0.0	2.3	10.9	1.6	0.0	0.4	2.0
blackberry	0.0	0.0	1.2	1.2	0.0	0.0	0.2	0.2
blueberry	0.2	0.1	0.0	0.3	0.0	0.0	0.0	0.0
cantaloupe	0.3	0.0	0.4	0.7	0.0	0.0	0.0	0.0
gooseberry	0.6	tr[e]	0.2	0.8	0.1	tr	0.0	0.1
grapes, black	0.5	0.0	0.6	1.1	0.1	0.0	0.1	0.2
grapes, Thompson	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
muskmelon	0.3	0.0	0.9	1.2	0.0	0.0	0.1	0.1
orange, navel	1.7	0.0	1.1	2.8	0.2	0.0	0.1	0.3
peach	3.5	0.0	0.0	3.5	0.4	0.0	0.0	0.4
pear, bosc	0.8	0.0	0.0	0.8	0.1	0.0	0.0	0.1
pear, d'Anjou	0.3	0.0	1.1	1.4	0.0	0.0	0.2	0.2
plantain	1.1	0.0	0.0	1.1	0.4	0.0	0.0	0.4
plum, red	1.8	0.2	0.0	2.0	0.2	0.0	0.0	0.2
rasberry, red	1.4	0.1	0.0	1.5	0.2	0.0	0.0	0.2
rhubarb	0.3	0.0	0.0	0.3	0.0	0.0	0.0	0.0
strawberry	tr	0.0	0.0	tr	tr	0.0	0.0	tr
watermelon	2.8	0.0	0.1	3.0	0.2	0.0	0.0	0.2

[a] 1-Kestose. [b] Nystose. [c] 1^F-β-Fructofuranosylnystose. [d] Total fructooligosaccharide. [e] tr, <12.5 ppm practical detection limit.

Table 2. Fructooligosaccharide Percent Recovery of Fruits

ingredient	GF_2[a]	GF_3[b]	GF_4[c]
apple, Red Delicious	100	107	111
apple, Golden Delicious	102	103	105
apple, Granny Smith	95	103	110
apple, Jonagold	98	101	106
apple, Rome	105	101	102
banana	98	107	103
banana, green	95	98	99
banana, red	102	103	101
banana, ripe	94	102	97
blackberry	102	104	91
blueberry	90	107	105
cantaloupe	92	98	109
gooseberry	106	102	96
grapes, black	117	106	105
grapes, Thompson	106	98	83
muskmelon	105	104	92
orange, navel	96	102	120
peach	100	106	92
pear, bosc	98	106	114
pear, d'Anjou	97	105	100
plantain	96	108	108
plum, red	87	100	97
rasberry, red	95	98	95
rhubarb	99	101	96
strawberry	107	96	119
watermelon	92	100	87

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

The FOS composition and percent recoveries for feedstuffs are presented in Tables 5 and 6, respectively. Of the grains analyzed, rye had the highest total FOS content of 4.1 mg/g of DM, while the remaining grains were at least 50% lower in FOS content. Since the grains were primarily dry, the total FOS content expressed on an as-is basis was slightly lower, with rye remaining as the grain with the highest total FOS followed by barley and wheat. Regarding the individual GF_n units, the GF_2 unit made up the largest percentage of total FOS, followed by GF_4 and GF_3 units for grains. Forages were low in total FOS, ranging from 0.0 (alfalfa and wheat straw) to 1.0 mg/g of DM (timothy hay).

Table 3. Fructooligosaccharide Composition of Vegetables

ingredient	mg/g of DM				mg/g of as is			
	GF_2[a]	GF_3[b]	GF_4[c]	total[d]	GF_2	GF_4	GF_4	total
acorn squash	1.4	0.0	1.9	3.3	0.2	0.0	0.2	0.4
artichoke, globe	13.4	5.5	2.8	21.8	1.5	0.6	0.3	2.4
asparagus	0.3	0.0	0.0	0.3	0.0	0.0	0.0	0.0
bean, green	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
bean, kidney	0.0	0.1	tr[e]	0.1	0.0	0.1	tr	0.1
beet, red	0.1	0.0	0.0	0.1	0.0	0.0	0.0	0.0
carrot, Bunny Luv	2.2	0.0	0.0	2.2	0.3	0.0	0.0	0.3
carrot, Dole	1.4	0.0	0.0	1.4	0.2	0.0	0.0	0.2
celery	0.2	0.0	0.4	0.6	0.0	0.0	0.0	0.0
chicory root, raw	9.1	6.1	5.9	21.0	1.7	1.1	1.1	3.9
chicory root, roasted	1.2	2.4	0.8	4.4	1.1	2.2	0.8	4.2
Chinese chive	0.4	0.3	0.4	1.1	0.0	0.0	0.0	0.0
daikon	0.5	0.0	0.0	0.5	0.0	0.0	0.0	0.0
eggplant	0.2	0.0	0.4	0.6	0.0	0.0	0.0	0.0
endive	0.1	0.0	0.2	0.3	0.0	0.0	0.0	0.0
garlic	8.7	1.2	0.4	10.3	3.3	0.4	0.2	3.9
garlic powder	0.8	0.6	0.3	1.7	0.7	0.6	0.3	1.6
ginger root	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Jerusalem artichoke	93.9	94.3	98.1	286.2	19.2	19.2	20.0	58.4
kiwi	0.1	0.0	0.0	0.1	0.0	0.0	0.0	0.0
leek	3.4	0.6	0.7	4.8	0.7	0.1	0.1	0.9
lettuce	4.9	1.9	1.1	7.9	0.3	0.1	0.1	0.5
onion, red	11.7	2.1	0.9	14.7	1.1	0.2	0.1	1.4
onion, Welch	5.8	3.9	3.6	13.4	0.5	0.3	0.3	1.1
onion, white	17.1	8.8	6.1	32.0	1.7	0.9	0.6	3.1
onion, yellow	15.5	6.7	4.2	26.4	1.5	0.6	0.4	2.6
onion powder	18.5	16.5	12.7	47.7	17.5	15.5	12.0	45.0
peas	0.2	0.2	0.4	0.7	0.0	0.0	0.1	0.1
peas, snap	0.5	0.0	8.0	8.4	0.1	0.0	1.0	1.1
peas, snow	1.0	0.0	5.4	6.5	0.1	0.0	0.5	0.6
potato, Idaho	0.2	0.0	0.0	0.2	0.0	0.0	0.0	0.0
potato, sweet	0.8	0.0	0.0	0.8	0.2	0.0	0.0	0.2
radish, red	0.0	0.0	3.0	3.0	0.0	0.0	0.1	0.1
shallot	28.2	14.2	10.6	52.9	4.5	2.3	1.7	8.5
taro root	0.1	0.0	0.1	0.1	0.0	0.0	0.0	0.0
tomato	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
tomato, cherry	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
tomato, Roma	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
yam	0.9	0.0	0.0	0.9	0.2	0.0	0.0	0.2
zucchini	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

[a] 1-Kestose. [b] Nystose. [c] 1^F-β-Fructofuranosylnystose. [d] Total fructooligosaccharide. [e] tr, <12.5 ppm practical detection limit.

Again, the forages had been dried; thus, the as-is data are slightly lower with a similar pattern as for grains. Within forages, no clear pattern existed for the individual GF_n units. The GF_2, GF_3, and GF_4 units were in similar amounts, depending upon individual forages.

Other feedstuffs also varied widely in total FOS content. The wheat sources were the highest, ranging from 4.0 to 5.1 mg of total FOS/g of DM for wheat bran and wheat middlings, respectively. Peanut hulls and alfalfa meal were moderate, with 2.4 and 2.2 mg of total FOS/g of DM, respectively. The remaining feedstuffs had much less FOS, with values <0.3 mg/g. These ingredients generally are fed as dried feedstuffs; therefore, the as-is values demonstrate a similar trend of being slightly lower than the DM values. Wheat sources were high in GF_2 units except for wheat germ, which had a high GF_4 content. Peanut hulls and alfalfa meal, which were moderate in total FOS, were primarily made up of GF_4 units, followed by GF_2 and GF_3 units. The remaining feedstuffs were generally composed of GF_2 units. The percent recoveries for the feedstuffs were similar to those of fruits and vegetables, ranging from 83 to 122%. The majority of the percent recovery values for feedstuffs were 100 $\pm$ 10%.

Several materials analyzed in the present study were also analyzed by an independent industrial laboratory (Ross Products Division, Columbus, OH) utilizing the

Table 4. Fructooligosaccharide Percent Recovery of Vegetables

ingredient	GF_2[a]	GF_3[b]	GF_4[c]
acorn squash	91	81	87
artichoke, globe	87	91	90
asparagus	94	90	99
bean, green	98	100	94
bean, kidney	103	101	107
beet, red	101	98	104
carrot, Bunny Luv	94	101	101
carrot, Dole	98	102	104
celery	103	100	104
chicory root, raw	82	99	106
chicory root, roasted	100	98	95
Chinese chive	108	108	104
daikon	99	99	105
eggplant	106	102	103
endive	105	104	112
garlic	91	95	95
garlic powder	103	100	99
ginger root	106	102	106
Jerusalem artichoke	88	93	103
kiwi	91	92	93
leek	89	100	99
lettuce	98	98	100
onion, red	99	103	109
onion, Welch	99	102	99
onion, white	100	106	104
onion, yellow	99	104	88
onion powder	94	100	100
peas	92	100	98
peas, snap	100	98	99
peas, snow	92	101	96
potato, Idaho	102	112	110
potato, sweet	100	99	108
radish, red	105	99	88
shallot	90	97	93
taro root	97	97	101
tomato	104	100	103
tomato, cherry	108	107	107
tomtao, Roma	104	108	123
yam	86	101	102
zucchini	92	92	112

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

Table 5. Fructooligosaccharide Composition of Feedstuffs

	mg/g of DM				mg/g as is			
ingredient	GF_2[a]	GF_3[b]	GF_4[c]	total[d]	GF_2	GF_3	GF_4	total
grains								
barley	1.5	0.4	0.0	1.9	1.3	0.4	0.0	1.7
corn	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
hominy	tr[e]	0.0	0.0	tr	tr	0.0	0.0	tr
milo	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
oats	0.4	0.0	0.0	0.4	0.3	0.0	0.0	0.3
rice, brown	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
rice, white	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
rye	3.0	0.5	0.6	4.1	2.8	0.5	0.6	3.8
soybean	0.0	tr	tr	tr	0.0	tr	tr	tr
wheat	1.1	0.1	0.2	1.4	1.0	0.1	0.2	1.3
forages								
alfalfa	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
bromegrass	0.0	tr	0.0	tr	0.0	tr	0.0	tr
clover hay	0.4	0.0	0.0	0.4	0.4	0.0	0.0	0.4
oat straw	0.0	0.2	0.6	0.8	0.0	0.2	0.5	0.7
orchardgrass	0.0	0.4	0.5	1.0	0.0	0.1	0.1	0.2
timothy hay	0.6	0.0	0.4	1.0	0.6	0.0	0.4	1.0
wheat straw	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
other								
alfalfa meal	0.1	0.0	2.1	2.2	0.1	0.0	2.0	2.1
beet pulp	0.1	0.0	0.0	0.1	0.1	0.0	0.0	0.1
brewer's rice	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
canola meal	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
corn distillers sol.	tr	0.0	0.0	tr	0.0	0.0	0.0	tr
corn gluten feed	0.0	0.1	0.0	0.1	0.0	0.1	0.0	0.1
corn gluten meal	0.0	0.2	0.1	0.3	0.0	0.2	0.1	0.3
oat groats	0.1	0.0	0.0	0.1	0.1	0.0	0.0	0.1
peanut hulls	0.3	0.1	2.1	2.4	0.2	0.1	1.9	2.2
rice bran	0.1	0.0	0.0	0.1	0.1	0.0	0.0	0.1
rice hulls	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
seaweed	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
soybean hulls	0.1	0.0	0.0	0.1	0.1	0.0	0.0	0.1
soybean meal	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
wheat bran	3.8	0.2	0.0	4.0	3.4	0.2	0.0	3.5
wheat germ	2.2	0.2	2.3	4.7	2.0	0.2	2.1	4.2
wheat middlings	5.1	0.0	0.0	5.1	4.6	0.0	0.0	4.6

[a] 1-Kestose. [b] Nystose. [c] 1^F-β-Fructofuranosylnystose. [d] Total fructooligosaccharide. [e] tr, <12.5 ppm practical detection limit.

same analytical method. Table 7 represents the results of FOS determination in processed foods from both laboratories. In general, the results compared very well, with an average RSD of 3.0%. The only discrepancy noted was the detection of small amounts of GF_4 units in the processed samples, dark brown sugar and tomato paste, by the industry laboratory and a larger difference between the laboratories for GF_2 units in garlic tablets compared to other analysis.

DISCUSSION

Foods and feeds that had been reported previously to contain FOS, and many others, were analyzed to characterize total FOS content and individual GF_n units. The HPLC method was able to accurately separate and quantitatively measure individual GF_n units. Compared to the HPLC method of Sims et al. (1991), the current method was shorter in time (60 vs 80 min) and required less sample preparation. Percent recoveries were primarily $100 \pm 10\%$ for all components; thus, individual GF_n units, regardless of sample source, would not be influenced significantly by recoveries. In contrast, Molis et al. (1996) determined percent recovery of FOS added to fecal samples to be $98 \pm 1\%$ recovered, which may be less variable compared to the current study due to one sample source analyzed. In comparison with other data, the present study yielded slightly lower values than those reported by Mitsuoka et al. (1987) and Tashiro et al. (1992) for onion, Welch onion, and garlic. This may be attributable to sampling technique, sample origin, and extraction. However, FOS concentrations reported by Spiegel et al. (1994) for banana, barley, garlic, onion, and rye are similar to those in the current study.

It is well-known that FOS is produced in the roots, tubers, and fruits of plants of the Compositae (Bacon and Edelman, 1951; Pollard and Amuti, 1981), Amaryllidadeae (Bacon, 1959; Darbyshire and Henry, 1978, 1981), Liliaceae (Shiomi et al., 1976), and Gramineae families (Nilsson et al., 1986; Saunders et al., 1975; Van Loo et al., 1995). Many samples in the present study were members of these plant families. Globe artichoke, Jerusalem artichoke, and chicory are Compositae species. Onions, garlic, leek, shallot, and Chinese chive are members of the *Allium* species in the Amaryllidadeae family. The Liliaceae are represented by asparagus, while the Gramineae family is represented by rye, wheat, barley, oat, and other grains exhibiting zero or trace amounts of FOS. The Compositae family had the most FOS on a DM basis, followed by the *Allium* species of the Amaryllidadeae family in the present study. Even though the present work did not detect FOS in raw tomatoes [as noted by Tashiro et al. (1992) and Spiegel et al. (1994)], quantifiable amounts of GF_2 were detected in tomato paste.

The ability to accurately quantify the FOS content of various foods and feeds allows for the estimation of average FOS intakes. Since FOS is naturally occurring, humans consume FOS daily as part of their regular diet.

Table 6. Fructooligosaccharide Percent Recovery of Feedstuffs

ingredient	GF_2[a]	GF_3[b]	GF_4[c]
grains			
barley	92	105	102
corn	104	103	100
hominy	105	103	110
milo	106	105	105
oats	98	102	111
rice, brown	96	95	95
rice, white	97	96	96
rye	86	102	99
soybean	116	104	107
wheat	94	98	95
forages			
alfalfa	97	87	83
bromegrass	102	90	91
clover hay	96	92	92
oat straw	98	97	104
orchardgrass	110	105	109
timothy hay	98	91	92
wheat straw	98	97	98
other			
alfalfa meal	100	99	97
beet pulp	106	107	102
brewer's rice	104	102	100
canola meal	105	89	92
corn distillers solubles	94	92	99
corn gluten feed	101	100	106
corn gluten meal	96	98	101
oat groats	103	96	96
peanut hulls	122	107	119
rice bran	103	100	102
rice hulls	102	102	99
seaweed	103	102	101
soybean hulls	111	108	102
soybean meal	99	102	99
wheat bran	100	105	106
wheat germ	84	99	85
wheat middlings	87	100	92

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

Table 7. Fructooligosaccharide Composition of Processed Foods

	mg/g as is							
	Ross Laboratories				University of Illinois			
foodstuff	GF_2[a]	GF_3[b]	GF_4[c]	total[d]	GF_2	GF_3	GF_4	total
Mega apple juice	tr[e]	tr	tr	tr	tr	tr	tr	tr
garlic plus FOS tablet[f]	122.0	117.0	18.0	257.0	121.0	115.0	19.0	255.0
infant formula[g]	8.4	8.2	1.2	18.0	7.7	7.7	1.2	17.0
minced onion[h]	17.0	16.0	11.0	44.0	16.0	15.0	11.0	42.0
sugar, dark brown	tr	tr	tr	1.2	0.9	tr	tr	0.9
tomato paste (Meijer)	tr	tr	2.2	2.5	0.4	tr	tr	0.4

[a] 1-Kestose. [b] Nystose. [c] 1^F-β-Fructofuranosylnystose. [d] Total fructooligosaccharide. [e] tr, <12.5 ppm practical detection limit. [f] Quintessence garlic plus FOS tablet. [g] Softcurd Powdered Formula F&P-f, Meiji Milk Co., Ltd. Tokyo. [h] McCormick minced onion.

It is estimated that the daily consumption of FOS by the North American population is between 2 and 8 g per person per day (Egan and Petersen, 1992). In the United States, the most commonly consumed foods containing FOS include globe artichoke, banana, garlic, onion, and several cereal grains. To evaluate the average daily exposure of the U.S. population to FOS through dietary consumption using the current FOS values, food consumption values were obtained from the EPA's Dietary Risk Evaluation System (DRES) database (EPA, 1984). To calculate the dietary exposure of FOS, the DRES consumption estimates were combined with the food-specific FOS concentrations measured in the present study. The DRES average daily food intake for globe artichoke, banana, wheat, rye, garlic, onion (red, white, yellow), leek, and barley were 3.21×10^{-3}, 2.28×10^{-1}, 1.41×10^{-1}, 2.74×10^{-4}, 7.62×10^{-4}, 1.08×10^{-1}, 3.88×10^{-5}, and 5.73×10^{-2} g/kg of body weight. The resulting estimates of average daily FOS intake from the average U.S. diet for globe artichoke, banana, wheat, rye, garlic, onion (red, white, yellow), leek, and barley were 7.67×10^{-6}, 3.24 x 10^{-4}, 1.79×10^{-4}, 1.04×10^{-6}, 2.96×10^{-6}, 2.53×10^{-4}, 3.84×10^{-7}, and 1.00×10^{-4} g/kg of body weight. Values are not corrected for percent recoveries. However, the actual average intake of FOS through the contemporary diet may be greater. As represented in the literature, FOS concentrations in foodstuffs tend to vary depending upon specific plant traits, harvest time, and storage time (Darbyshire, 1978; Suzuki and Cutcliffe, 1989). Therefore, actual consumption levels are subject to variability.

In conclusion, ion exchange HPLC with pulsed electrochemical detection is a viable means for quantifying FOS content of foods and feeds. The method provides excellent separation, recovery, and detection of FOS compared to other HPLC techniques. Furthermore, this comprehensive database on FOS content may better facilitate nutritional formulation and diet selection for higher FOS consumption.

LITERATURE CITED

Association of Official Analytical Chemists. In *Official Methods of Analysis*, 14th ed.; AOAC: Washington, DC, 1984.

Bacon, J. S. D. The Trisaccharide Fraction of Some Monocotyledons. *Biochem. J.* **1959**, *73*, 507−514.

Bacon, J. S. D.; Edelman, J. The Carbohydrates of the Jerusalem Artichoke and Other Compositae. *Biochem. J.* **1951**, *48*, 114−126.

Clevenger, M. A.; Turnbul, D.; Inone, H.; Enomoto, M.; Allen, J. A.; Henderson, L. M.; Jones, E. Toxicological Evaluation of Neosugar: Genotoxicity, Carcinogenicity, and Chronic Toxicity. *J. Am. Col. Toxicol.* **1988**, *7*, 643−662.

Darbyshire, B. Changes in the Carbohydrate Content of Onion Bulbs Stored for Various Times at Different Temperatures. *J. Hortic. Sci.* **1978**, *53*, 195−201.

Darbyshire, B.; Henry, R. J. The Distribution of Fructans in Onions. *New Phytol.* **1978**, *81*, 29−34.

Darbyshire, B.; Henry, R. J. Differences in Fructan Content and Synthesis in Some *Allium* Species. *New Phytol.* **1981**, *87*, 249−256.

Egan, S. K.; Petersen, B. J. *Estimated Consumption of Inulin and Oligofructose by the U.S. Population*; Technical Assessment Systems Inc.: Washington, DC, 1992.

EPA. Tolerance Assessment System. Office of Pesticide Programs, Hazard Evaluation Div. Toxicology Branch, Environmental Protection Agency: Washington, DC, 1984.

Gibson, G. R.; Roberfroid, M. B. Dietary Modulation of the Human Colonic Microbiota: Introducing the Concept of Prebiotics. *J. Nutr.* **1995**, *125*, 1401−1412.

Hidaka, H.; Eida, T.; Takizawa, T.; Tokanaga, T.; Tashiro, Y. Effects of Fructooligosaccharides on Intestinal Flora and Human Health. *Bifidobact. Microflora* **1986**, *5*, 37−50.

Hidaka, H.; Hirayama, H.; Sumi, N. A Fructooligosaccharide-Producing Enzyme from *Aspergillus niger* ATCC 20611. *Agric. Biol. Chem.* **1988**, *52*, 1181−1187.

Hidaka, H.; Hirayama, H.; Yamada, K. Fructooligosaccharides: Enzymatic Preparation and Biofunctions. *J. Carbohydr. Chem.* **1991**, *10*, 509−522.

Hirayama, M.; Sumi, N.; Hidaka, H. Purification and Properties of a Fructooligosaccharides-Producing β-fructofuranosidase from *Aspergillus niger* ATCC 20611. *Agric. Biol. Chem.* **1989**, *53*, 667−673.

Lewis, D. H. Nomenclature and Diagrammatic Representation of Oligomeric Fructans—A Paper for Discussion. *New Phytol.* **1993**, *124*, 583−594.

Mitsuoka, T.; Hidaka, H.; Eida, T. Effect of Fructo-oligosaccharides on Intestinal Microflora. *Nahrung* **1987**, *31*, 427−436.

Molis, C.; Flourié, B.; Ouarne, F.; Gailing, M. F.; Lartigue, S.; Guibert, A.; Bornet, F.; Galmiche, J. P. Digestion, Excretion, and Energy Value of Fructooligosaccharides in Healthy Humans. *Am. J. Clin. Nutr.* **1996**, *64*, 324−328.

Nilsson, U.; Dahlqvist, A.; Nilsson, B. Cereal Fructosans: Part 2—Characterization and Structure of Wheat Fructosans. *Food Chem.* **1986**, *22*, 95−106.

Pollard, C. J.; Amuti, K. S. Fructose Oligosaccharides: Possible Markers of Phylogenetic Relationships Among Dicotyledonous Plant Families. *Biochem. Syst. Ecol.* **1981**, *9*, 69−78.

Roberfroid, M.; Gibson, G. R.; Delzenne, N. The Biochemistry of Oligofructose, A Nondigestible Fiber: An Approach to Calculate its Caloric Value. *Nutr. Rev.* **1993**, *51*, 137−146.

Saunders, R. M.; Betschart, A. A.; Lorenz, K. The Sugars of Triticale Bran. *Cereal Chem.* **1975**, *52*, 472−478.

Shiomi, N.; Yamada, J.; Izawa, M. Isolation and Identification of Fructo-oligosaccharides in Roots of Asparagus (*Asparagus officinalis* L.). *Agric. Biol. Chem.* **1976**, *40*, 567−575.

Sims, I. M.; Smouter, H.; Pollock, C. J.; Simpson, R. J. The Separation of Complex Mixtures of Fructo-oligosaccharides from Plants. *Plant Physiol. Biochem.* **1991**, *29*, 257−267.

Spiegel, J. E.; Rose, R.; Karabell, P.; Frankos, V. H.; Schmitt, D. F. Safety and Benefits of Fructooligosaccharides as Food Ingredients. *Food Technol.* **1994**, *48*, 85−89.

Sukuki, M.; Cutcliffe, J. A. Fructans in Onion Bulbs in Relation to Storage Life. *Can. J. Plant Sci.* **1989**, *69*, 1327−1333.

Tashiro, Y.; Eida, T.; Hidaka, H. Distribution and Quantification of Fructooligosaccharides in Food Materials. *Sci. Rep. Meiji Seiki Kaisha* **1992**, *31*, 35−40.

Tokunaga, T.; Oku, T.; Hosoya, N. Influence of Chronic Intake of New Sweetener Fructooligosaccharide (Neosugar) on Growth and Gastrointestinal Function of the Rat. *J. Nutr. Sci. Vitaminol.* **1986**, *32*, 111−121.

Tokunaga, T.; Oku, T.; Hosoya, N. Utilization and Excretion of a New Sweetener, Fructooligosaccharide (Neosugar) in Rats. *J. Nutr.* **1989**, *119*, 553−559.

Tomomatsu, H. Health Effects of Oligosaccharides. *Food Technol.* **1994**, *48*, 61−65.

Van Loo, J.; Coussement, P.; De Leenheer, L.; Hoebregs, H.; Smits, G. On the Presence of Inulin and Oligofructose as Natural Ingredients in the Western Diet. *Crit. Rev. Food Sci. Nutr.* **1995**, *35*, 525−552.

Received for review January 27, 1997. Revised manuscript received May 21, 1997. Accepted May 22, 1997.[⊗]

JF970087G

[⊗] Abstract published in *Advance ACS Abstracts,* July 15, 1997.