SPECIAL GUEST EDITOR SECTION

Determination of Oligofructose, a Soluble Dietary Fiber, by High-Temperature Capillary Gas Chromatography

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A high-temperature capillary gas chromatographic method was developed for the quantitative determination of oligofructose in foods and food products. Sample preparation involves oxymation and silylation of the extracted sugars. The oximetrimethylsilyl derivatives are analyzed on an apolar capillary column, with detection by flame ionization. The method is accurate, with recovery of spiked samples at >96%. Repeatability was excellent; RSD values of 1.1% were obtained. Other common oligosaccharides, such as malto-, isomalto-, and galactooligosaccharides, and levan do not interfere, making the method specific and reliable.

Oligofructose, along with inulin, belongs to the fructan group. Its basic molecular structure consists of primarily (2 → 1)-linked β-D-fructofuranosyl units, possibly terminating in glucose, and represented by GF_n and F_m with G = glucose and F = fructose, *n* from 2 to 9, and *m* from 2 to 10 (1). Oligofructose is present in significant amounts in common fruits and vegetables (2). It is a natural constituent of inulin and is obtained industrially by partial enzymatic hydrolysis of inulin (1).

Oligofructose (OFS) is a soluble dietary fiber. Like inulin, it is metabolized in the same way as dietary fibers and shows significant dietary fiber effects (3, 4).

Because of its solubility in ethanol–water (4 + 1), oligofructose is not included as part of the fiber fraction used in current dietary fiber analytical methods. It can be quantitated by using AOAC Official Method **997.08** (5), which relies on enzymatic treatment of the sample with inulinase followed by high pressure anion exchange chromatography with pulsed amperometric detection (HPAEC–PAD) determination of released sugars. Durgnat and Martinez (6) identified and quantitated OFS in commercial foods by using HPAEC–PAD and by comparison with the corresponding raw material. With cation-exchange liquid chromatography (LC), some OFS compounds coelute and other oligosaccharides such as malto-n-ose may interfere.

Capillary gas chromatography (CGC) may be more complex than LC and HPAEC because of the comprehensive sample pretreatment to derivatize the sugars. However, high temperature CGC is a very robust and productive analytical technique which measures sugars up to DP (degree of polymerization) 10 in complex sample matrixes in only one chromatographic run. Our procedure involves oxymation and silylation of the extracted sugars (7). The oxime-trimethylsilyl derivatives are extracted using isooctane; cool on-column injections are performed on an apolar capillary Al-clad column. The temperature program rises to 440°C, with detection by flame ionization.

METHOD

Apparatus

(**a**) *High temperature gas chromatograph*.—Carlo-Erba (Rodina, Italy) HRGC 5300-HT equipped with a cool on-column auto-injector AS-550 and a flame ionization detector or equivalent. The GC instrument is equipped with an Al-clad capillary column, 6 m long × 0.53 mm id, and coated with 0.1 μm film of 5% phenyl polycarborane-siloxane (SGE). The oven temperature was programmed from 105 to 440°C at 10°/min. Helium was used as carrier gas at a constant flow of 9 mL/min. The detector temperature was set at 447°C, with hydrogen at 60 kPa, air at 110 kPa, and nitrogen as make-up gas at 60 kPa. Sample volumes injected were 1.0 μL. Parameters may vary to optimize the chromatography.

(**b**) *Sample preparation tubes*.—16 × 100 mm reaction tubes with Teflon-lined screw caps.

(**c**) *Vortex mixer*.—Labinco (Breda, The Netherlands) type L 24, or equivalent.

(**d**) *Desiccator*.—With SiO_2 or equivalent desiccant. Bi-weekly, dry desiccant overnight at 130°C.

(**e**) *Data integration system*.—Chromperfect (Justice Innovations, Palo Alto, CA), or equivalent.

(**f**) *Glass bottles*.—100 mL, with screw caps, Duran Schott (Mainz, Germany), or equivalent.

(**g**) *Balance*.—Analytical, sensitivity ± 0.01 mg.

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Table 1. Determination of oligofructose (OFS) in spiked jam and yogurt with jam

Spiked amount OFS, g/100 g	OFS, g/100 g	Recovery, %
	Jam 1	
0.0	0.19	
3.05	3.12	96.3
8.89	8.83	97.2
16.2	16.0	97.6
	Jam 2	
0.0	0.16	
8.10	8.04	97.3
14.0	13.8	97.5
	Yogurt with jam	
0.0	0.0	
3.25	3.19	98.2
3.45	3.39	98.3

Reagents

Use deionized water throughout.

(**a**) *Hydroxylamine-HCl*.—Analytical grade (No. 455461, Carlo-Erba). Dissolve 2.5 g in 50 mL dry pyridine. The solution is stable for 1 month when stored in refrigerator.

(**b**) *Pyridine*.—Analytical grade (No. 27530, Pierce Chemical Co., Rockford, IL).

(**c**) *Trimethylsilylimidazole*.—Analytical grade (No. 88625, Pierce). Store in a refrigerator under nitrogen.

(**d**) *Glucose*.—D(+)-Dextrose anhydrous, reagent p.a. Dry sugar standard under reduced pressure at 55 ± 3°C for 48 h.

(**e**) *Fructose*.—Levulose, reagent p.a. Dry sugar standard under reduced pressure at 55 ± 3°C for 48 h.

(**f**) *Sucrose*.—Reagent p.a. Dry sugar standard under reduced pressure at 55 ± 3°C for 48 h.

(**g**) *Raffinose*.—Raffinose-pentahydrate p.a. (Vel-No. 1672, Merck-Eurolab, Leuven, Belgium).

(**h**) *Glucoheptose*.—D-Gluco-gulo-heptose (Pfanstiehl Laboratories, Waukegan, IL), No. G-111, or equivalent.

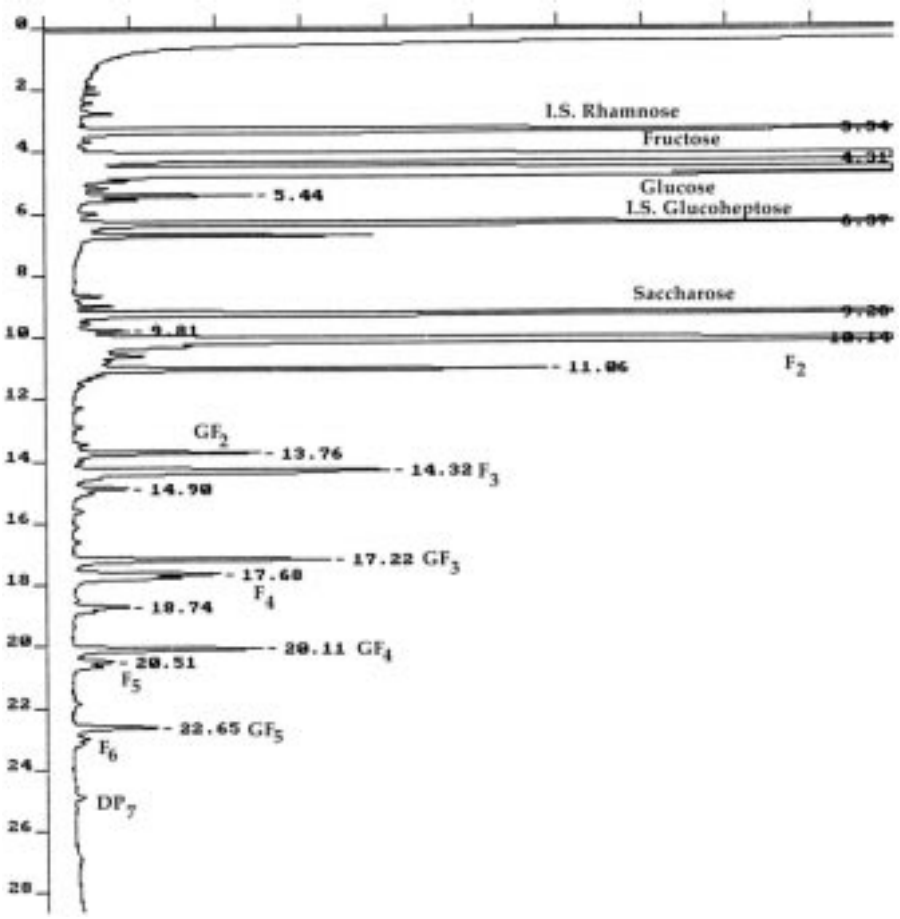

Figure 1. Analysis of fruit preparation with oligofructose content = 0.6 g/100 g.

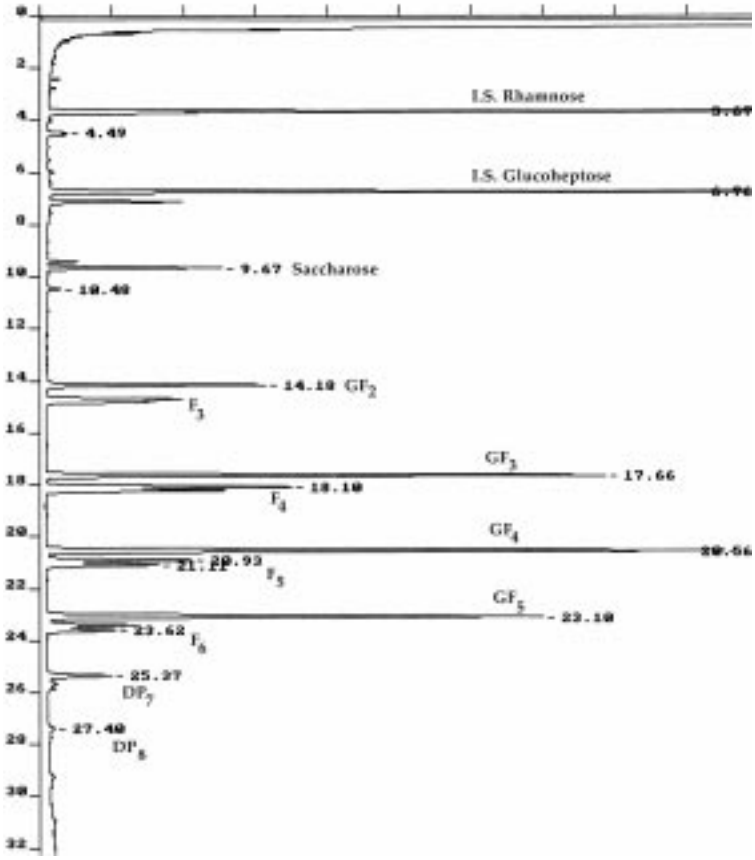

Figure 2. Analysis of Raftilose P95 X.

Table 2. Statistical data from high temperature CGC results of Raftilose P95X reference sample

Compound	Mean[a]	SD_r[b]	RSD_r[c]
Fructose	0.48	0.08	17
Glucose	0.04	0.01	23
Saccharose	3.49	0.07	2.1
F2	0.29	0.02	7.8
GF2	5.11	0.07	1.3
F3	5.52	0.06	1.2
GF3	15.2	0.18	1.2
F4	11.6	0.16	1.4
GF4	20.9	0.29	1.4
F5	7.57	0.19	2.6
GF5	16.4	0.25	1.5
F6	5.10	0.24	4.8
GF6	2.30	0.15	6.7
F7	0.64	0.06	9.0
DP8[d]	0.50	0.08	17
DP9	0.28	0.05	18
Sum sugars	95.5	0.93	1.0
Sum FOS	91.5	0.95	1.0

[a] Mean; g/100 g.
[b] SD_r, repeatability standard deviation.
[c] RSD_r repeatability relative standard deviation.
[d] DP, degree of polymerization.

Standardization

With each series of samples, 4 standard solutions are analyzed to calculate the response factors and draw a calibration graph for calculation of concentrations in real samples. Components used in standard solutions are fructose, glucose, sucrose, and raffinose, at concentrations of 5, 10, 20, and 30 mg, respectively, in 50 mL water. Gluco-gulo-heptose (20 mg) is added to the solutions as internal standard.

The other compounds of interest, kestose (GF_2), nystose (GF_3) to GF_6, and inulobiose (F_2) to inuloheptose (F_7) are not commercially available for standardization. The series from maltotriose to maltoheptose is no longer commercially available. Using this series of malto-n-ose standard sugars, we calculated the response factors of the oligofructose compounds of interest by interpolation. With information from unpublished studies on prep-HPAEC–PAD, we standardized the composition of a pure reference oligofructose sample, Raftilose® P95X. Two different concentrations of that reference sample were analyzed, together with the 4 standard sugar solutions for each series of samples. A study has been initiated to fractionate and purify the different oligofructose compounds for better standardization.

Sample Preparation

Homogenize samples immediately before analysis. For fruits, vegetables, cereal products, and processed foods, mix with interruptions in a blender for 2–10 min. Freeze sticky or

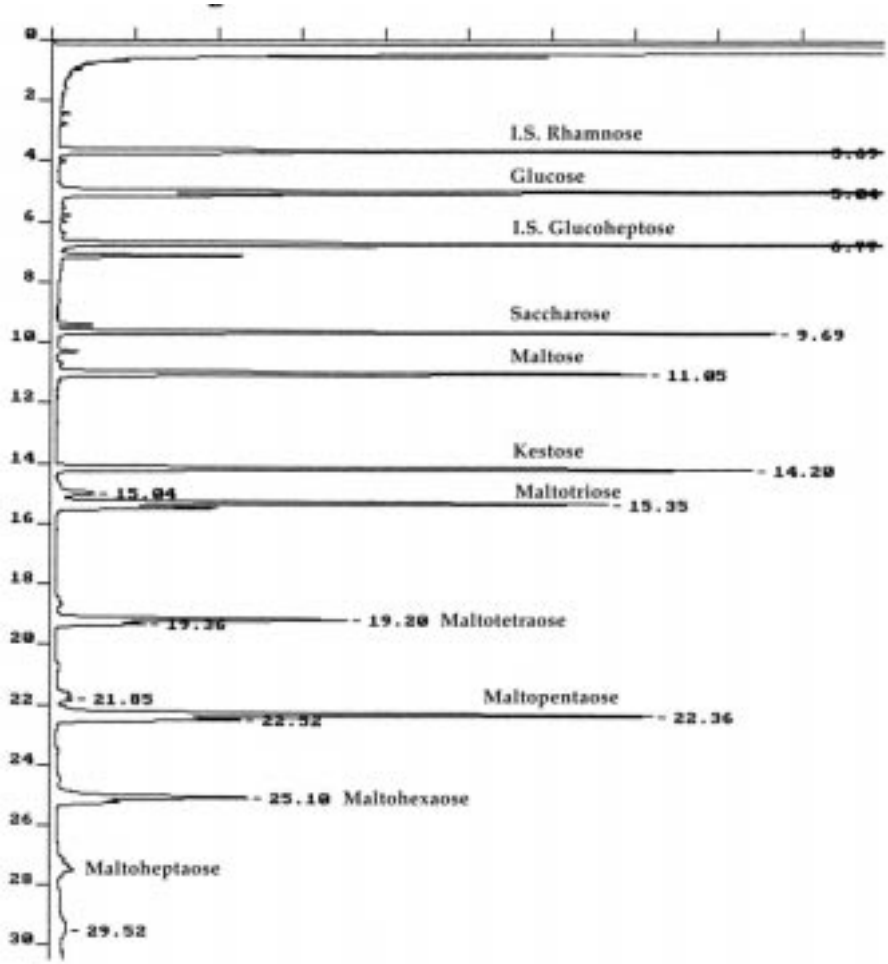

Figure 3. Analysis of maltooligosaccharides.

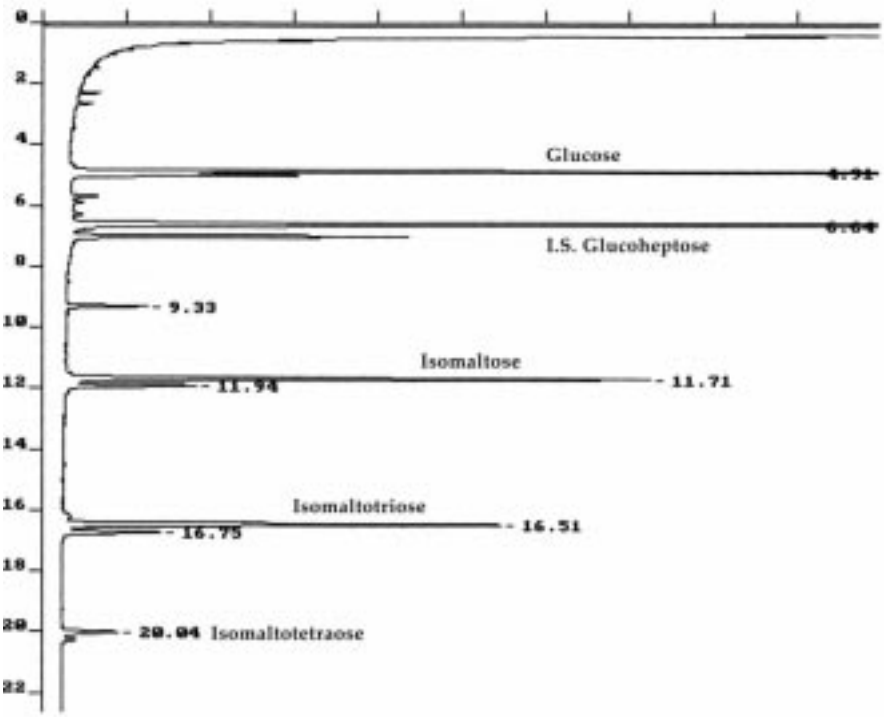

Figure 4. Analysis of isomaltooligosaccharides.

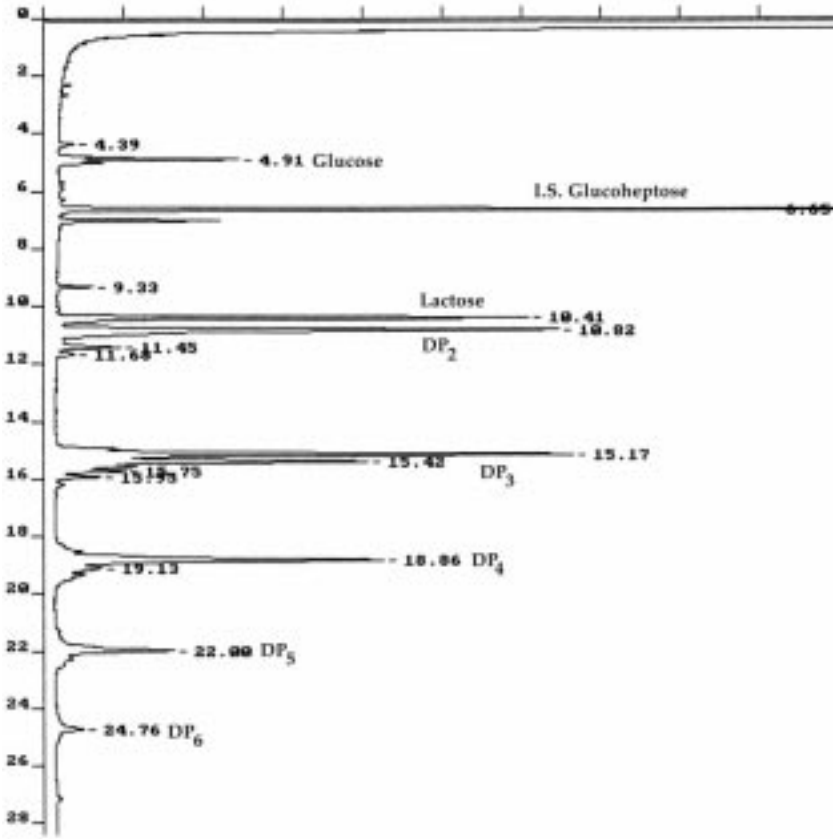

Figure 5. Analysis of galactooligosaccharides.

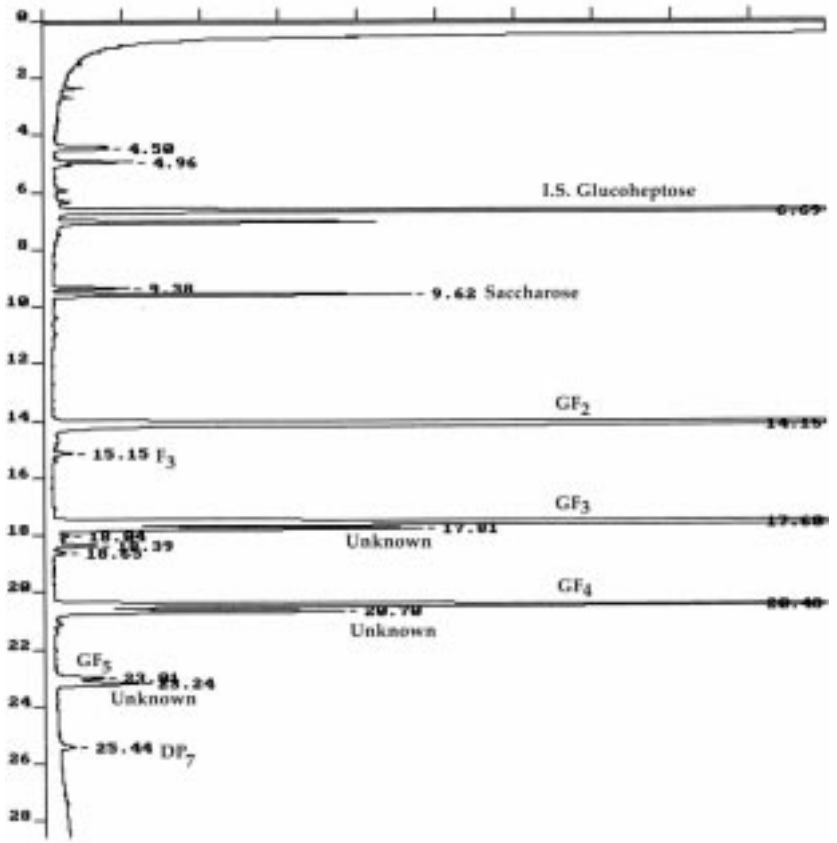

Figure 6. Analysis of Actilight.

fatty samples (chocolate and bars that form a paste-like mass) before mixing in blender. Cut gummy, sticky, and paste-like samples that cannot be mixed in blender, into small pieces with a knife or scissors so that no particle size is larger than 100 mm^3. Hard samples such as hard candies should be shattered in a mortar so that no particle is larger than 100 mm^3.

Extraction

Accurately weigh an amount of sample, corresponding to ca 150 mg oligofructose, but not exceeding 15 g sample, to ± 0.1 mg in a tared 100 mL glass bottle with screw cap. Add 20 mg internal standard, accurately weighed to ± 0.01 mg. Add ca 40 mL water. Measure pH immediately under mild agitation; pH should be between 5.0 and 9.0. If necessary, adjust pH immediately with 0.05N KOH or 0.05N HCl. This must be done quickly and accurately for as long as it takes to dissolve the sample completely. Rinse the electrode with water. Adjust to total volume of ca 50 mL, weigh, and homogenize.

For samples such as cakes or chocolate containing fats, transfer ca 4 mL solution in a reaction tube and add 4 mL hexane. Shake for 10 min on a Vortex mixer, and centrifuge to separate the organic phase. Discard the organic phase with a Pasteur pipet. Add another 4 mL hexane to repeat extraction. Centrifuge and discard the hexane phase.

Drying Procedure

Transfer 200 μL of samples or standard solutions in glass sample preparation tubes and dry before derivatization. Dry by one of the following procedures: (*1*) Place sample tubes in vacuum desiccator containing silica gel for at least 4 h. (*2*) Add ca 200 μL dichloromethane, mix on a Vortex mixer, and dry sample to constant volume at maximum 30°C under nitrogen stream; repeat the procedure to complete dryness.

Derivatization

The sugars are derivatized into volatile oxime-trimethylsilyl derivatives by a 2-step procedure and extraction into an organic solvent.

Transfer 200 μL oximation reagent to the sample tube in a dry syringe. Mix well for 10 min on a Vortex mixer and heat sample tube at 60°C for 15 min. Centrifuge 5 min and dry under nitrogen. Transfer 400 μL silylating agent to sample tube in a dry syringe and mix well on a Vortex mixer for 10 min.

Add 1000 μL water to sample tube to neutralize excess trimethylsilylimidazole (TSIM), add 2000 μL isooctane to sample tube, mix on Vortex mixer for 3 min, and centrifuge 5 min. Transfer isooctane to an injection vial and inject 1 μL.

In the autosampler, place a vial containing isooctane for each of 4 samples to flush.

Results and Conclusions

The performance of the method is illustrated with emphasis on accuracy, repeatability, and possible interference of other sugar compounds.

The accuracy was determined by the standard addition method. To 2 jam samples and to a yogurt with jam, all with very low OFS content, known amounts of Raftilose P95X were added. The original and the spiked samples were analyzed by the high temperature CGC method described (Table 1). The data obtained for the 7 spiked samples were excellent, showing a recovery >96%. Figure 1 shows a chromatogram of a fruit prepared with 0.6% OFS.

Two different concentrations of a Raftilose P95X reference sample were analyzed with each series of samples. Over 2 months, the reference sample was analyzed 17 times; the last 5 chromatographic analyses were performed after installation of a new capillary column. An example of a chromatogram is given in Figure 2.

The repeatability of the method was determined using these results. Statistical data are presented in Table 2. The relative standard deviation (RSD) for total OFS content was 1.1%. Repeatability for quantitation of individual compounds was very good: the RSD for GF_2, F_3, GF_3, F_4, GF_4, F_5, and GF_5 individually was >2.5%. The RSD for repeatability of compounds that represent <0.5% of the total OFS content was high, about 20%, but the peaks were small and the influence on the total precision was very limited.

To exclude possible interference from other sugar compounds, most common oligosaccharides were analyzed by the same method and with the same chromatographic parameters. Figures 3–5 show chromatograms of malto-, isomalto-, and galactooligosaccharide, respectively. None of the analyzed sugars coeluted with an oligofructose compound.

Figure 6 shows the chromatogram of Actilight, an enzymatically synthesized OFS. Unidentified peaks appear between the GF_n and the F_m compounds.

In conclusion, the method proved to be accurate and repeatable, and was not disturbed by interferences from other oligosaccharides.

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