Inulin Determination for Food Labeling

Angela Zuleta and María E. Sambucetti*

Faculty of Pharmacy and Biochemistry, University of Buenos Aires, Junín 956, (1113) Buenos Aires, Argentina

Inulin and oligofructose exhibit valuable nutritional and functional attributes, so they are used as supplements as soluble fiber or as macronutrient substitutes. As classic analytical methods for dietary fiber measurement are not effective, several specific methods have been proposed. These methods measure total fructans and are based on one or more enzymatic sample treatments and determination of released sugars. To determine inulin for labeling purposes, we developed an easy and rapid anion-exchange high-performance liquid chromatography (HPLC) method following water extraction of inulin. HPLC conditions included an Aminex HPX- 87C column (Bio-Rad), deionized water at 85 °C as the mobile phase and a refractive index detector. The tested foods included tailor-made food products containing known amounts of inulin and commercial products (cookies, milk, ice creams, cheese, and cereal bars). The average recovery was 97%, and the coefficient of variation ranged from 1.1 to 5% in the food matrixes. The obtained results showed that this method provides an easier, faster and cheaper alternative than previous techniques for determining inulin with enough accuracy and precision for routine labeling purposes by direct determination of inulin by HPLC with refractive index detection.

Keywords: *Inulin determination; food labeling; fructans*

INTRODUCTION

Inulin and oligofructose are mixtures of β (2→1) linked fructans; in most chains the terminal sugar is glucose. The degree of polymerization ranges between 3 and 60 and between 2 and 7 for inulin and oligofructose, respectively (*1*). Because of their structure, these compounds are not digested by the alimentary enzymes of man, so that they are considered fiber in most countries. Among their nutritional attributes, these substances stimulate beneficial gut microflora (*1*) and relieve constipation (*2*), as well as improving calcium availability (*3*). A potential reduction of cancer risk has also been advanced (*4*). Their calorie value is estimated to be 1.5 kcal/g (*5*). Functional properties such as solubility, water retention capacity and taste allow their use as macronutrient substitutes and or as soluble fiber supplements (*6*).

Because of its longer chain length, inulin is able to form inulin microcrystals when sheared in water or milk that are not discretely perceptible in the mouth but that interact to form a smooth creamy texture and provide a fat-like mouthfeel. For these reasons, inulin is an ingredient commonly used in biscuits and cakes, cereal bars, ice creams and desserts, milk, yogurt, and cheese (*6*).

The classic analytical method for dietary fiber analysis, AOAC International Method 985.29 (*7*) is not suitable for measuring inulin or oligofructose because these substances are partially precipitated in the alcohol treatment step. As a result several specific methods have been developed for their determination (*8*−*11*). These methods measure total fructans and are based on one or several enzymatic treatments of the sample and the determination of released sugars by different techniques, including high-performance anion-exchange chromatography with pulsed amperometric detection (HPAECC−PAD), fructose as reducing sugar by the *p*-hydroxybenzoic acid hydrazide (PAHBAH) method, gas−liquid chromatography (GLC) and high-performance liquid chromatography with refractive index detection (HPLC−RI), respectively. Fructan content is then calculated as the difference between the sugar contents before and after hydrolysis.

To determine inulin for labeling purposes, we developed an easy and rapid HPLC method, involving water extraction of inulin and its direct determination by HPLC−RI without hydrolysis.

MATERIALS AND METHODS

Reference sugars (glucose, fructose, galactose, lactose, maltose and sucrose) were from Sigma (carbohydrates kit, CAR-11); reference inulin was from Sigma (I-3754); and commercial inulin as Raftiline HP and Raftiline ST were from Orafti (Belgium).

The tested foods were (a) tailor-made food products containing known amounts of Raftiline HP, including sweet cookies, salted cookies made with whole meal, fermented milk, and skim milk; and (b) commercial products including milk, ice cream (three flavors), cheese, and cereal bars.

Sample Preparation. Samples were homogenized immediately before analysis. When solid, they were ground to pass a 0.5-mm sieve. In dairy products, the analyses were made on the whey obtained by the precipitation of protein with a few drops of acetic acid, followed by adjustment of the pH to 6.5.

Procedure. The sample (1 g) was accurately weighed into a 200-mL Pyrex beaker, treated with ca. 100 mL of boiling water at pH 6−8, and kept at 85 °C with continuous magnetic stirring on a hot plate for 15 min. It was then cooled to room temperature, the volume was made up to 100 mL, and the solution was filtered through a 0.20-μm membrane filter (Minisart Sartorius or equivalent) before injection. If the filtrate is kept refrigerated before analysis, the fructan tends to precipitate from solution, so it should be reheated to 80 °C and allowed to cool to room temperature before analysis.

10.1021/jf010505o CCC: $20.00
Published on Web 09/25/2001

The chromatographic equipment consisted of a Waters 6000A pump system, a Waters injector with a 50-μL sample loop, a refractive index detector (Waters R40), and an integrator (Data Module Waters). HPLC conditions were an Aminex HPX-87C (Bio-Rad) anion-exchange column, with deionized water at 85 °C as the mobile phase at a flux rate of 0.6 mL/min.

Calibration curves were plotted with 0.005−1 g/100 mL of inulin. To identify other sugars commonly present in samples, reference solutions with 1 g/100 mL of mono- and disaccharides were tested.

All determinations were made in triplicate.

RESULTS AND DISCUSSION

Most commercially available inulin is extracted from chicory roots (*Cichorium intybus*). The degree of polymerization (DP) varies as a function of the harvest date, but after extraction using a hot-water diffusion process, the resulting product, i.e., standard inulin (Raftiline ST), has an average DP of 10−12 with a 6−10% content of free sugars as sucrose, fructose, and glucose. A high-performance type of inulin (Raftiline HP), which provides better mimetic fat properties than standard inulin, is commonly used in the food industry. This product has an average DP of 25, and its molecular distribution ranges from 11 to 60 without sugars or polymers smaller than 11 DP (*6*).

The commercially available inulin from Sigma used as a standard in this work is from *Dahlia tubers*. This product is standardized to have an average DP of 27−29 (*12*).

The chromatographic system proposed herein employs a column widely used in food analysis to determine sugars and related components such as sorbitol and Polydextrose (*13*), which allows inulin to be isolated with a readily identifiable peak.

Figure 1A shows the chromatogram of a reference inulin, glucose, fructose, and disaccharide (lactose, maltose, or sucrose) mixture. This column does not allow diverse disaccharides to be separated. Commercial inulin Raftiline HP (C) as well as Sigma inulin (B) present only one peak with the same elution time. Despite its smaller average DP, Raftilin ST (D) shows a main peak that coincides with the Sigma and Raftiline HP peaks; however, a minor peak of sucrose appears, accounting for 5 g/100 g inulin. Quemener et al. determined values of 5.4 g/100 g, 1 g/100 g and 0.1 g/100 g of sucrose, fructose, and glucose, respectively (*14*). Such low concentrations of fructose and glucose are below the sensitivity of this method.

To test HPLC response linearity, we plotted area versus increasing amounts of reference inulin and obtained a correlation coefficient of $r^2 = 0.99$. The minimum detection level was 0.005 g/100 mL.

Figure 2 shows chromatograms of foods with various matrixes with or without inulin, all lacking any interfering peaks.

Assay accuracy was demonstrated by inulin recovery at different levels. Table 1 shows results in tailor-made foods with inulin contents ranging from 0.35 g/100 g to 6 g/100 g. Recoveries ranged from 96 to 98%, with an average value of 97% despite the different food matrixes. For commercial foods (Table 2), the average recovery was the same (97%), but the values ranged from 92 to 107%. In this type of food, recovery values were calculated from the content claimed on the label or personal communication from the producer, which would explain the greater dispersion of the recovery values. Although

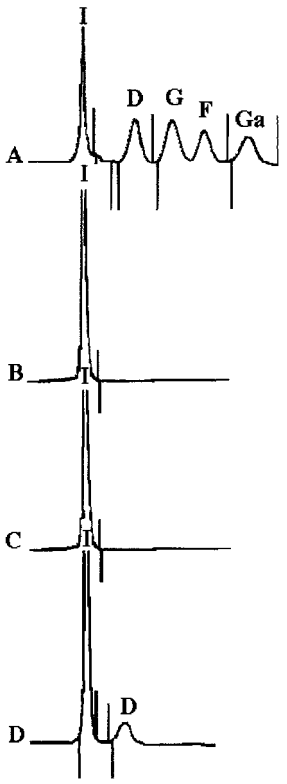

Figure 1. HPLC separation of sugars. (A) I, inulin; D, disaccharides; G, glucose; F, fructose; and Ga, galactose from Sigma. (B) I, inulin from Sigma. (C) I, Raftiline HP. (D) I, Raftiline ST.

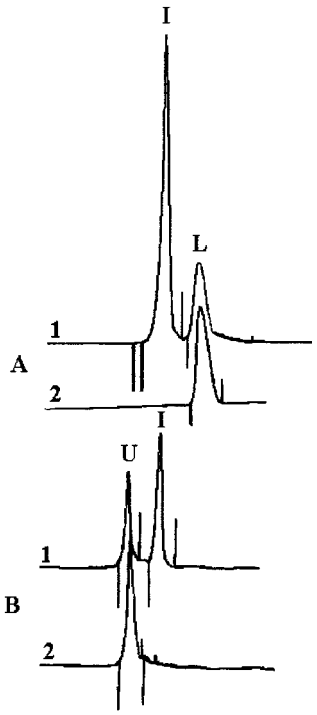

Figure 2. HPLC separation of sugars. (A) skim milk with (1) or without (2) Raftiline HP; I, inulin; L, lactose. (B) salted cookies with (1) or without (2) Raftiline HP; I, inulin; U, unknown.

analyzed samples contained Raftiline HP lacking free sugars, even when Raftiline ST, which contains free sugars, was used, the error committed with this method would be similar to that obtained by employing the AOAC International Method 997.08, because the free

Table 1. Inulin Recovery from Tailor-Made Foods with Different Matrixes

food	added (%)	found[a] (%)	recovery (%)	CV (%)
sweet cookies	0.35	0.34 ± 0.015	97	4.5
salted cookies	0.55	0.53 ± 0.014	96	2.6
skim milk	1.00	0.97 ± 0.011	97	1.1
fermented food (milk + inulin)	6.00	5.85 ± 0.100	98	1.7

[a] Mean ± SD of three measurements.

Table 2. Inulin Content in Commercial Food Samples

food	claimed (%)	found[a] (%)	recovery (%)	CV (%)
ice creams				
lemon	5.22	5.09 ± 0.23	97	4.5
vanilla	5.37	5.20 ± 0.26	97	5.0
chocolate	5.36	4.91 ± 0.25	93	5.0
cereal bar	18.00	16.57 ± 0.53	92	3.2
diet cheese	1.50	1.61 ± 0.02	107	1.2

[a] Mean ± SD of three measurements.

sugar quantification in the sample would be increased and so the resulting inulin value obtained by the difference before and after fructans hydrolysis would be lower.

The assay precision in the different food matrixes expressed as coefficient of variation ranged from 1.1 to 5%. This intralaboratory variation is similar or even smaller than the repeatability relative standard deviation (RSDr) obtained by methods that determine fructans as fructose that have obtained First Approval status from AOAC International Methods 997.08 and 999.03 (*8*, *9*).

We can therefore conclude that the results achieved herein support our method as an easy, fast and inexpensive alternative for determining inulin with sufficient accuracy and precision for routine labeling purposes.

LITERATURE CITED

(1) Roberfroid, M. B. The bifidogenic nature of chicory inulin and its hydrolysis products. *J. Nutr.* **1998**, *128*, 11−19.

(2) Hidaka, H.; Adachi, T.; Hirayama, M. Development and Beneficial Effects of Fructo-oligosaccharides (Neosugar). In *Advanced Dietary Fibre Technology*; McCleary B. V.; Prosky L., Eds. Blackwell Science Ltd.: London, 2001; pp 474−476.

(3) Van den Heuvel, E.; Muys, T.; Van Dokkum, W.; Schaafsma, G. Oligofructose stimulates calcium absorption in adolescents. *J. Am. Clin. Nutr.* **1999**, *69*, 544−548.

(4) Reddy, B. S. Possible mechanisms by which pro- and prebiotics influence colon carcinogenesis and tumor growth. *J. Nutr., Suppl. 7* **1999**, *129*, 1478S−1482S.

(5) Roberfroid, M. B. Caloric value of inulin and oligofructose. *J. Nutr., Suppl 7* **1999**, *129*, 1436S−1437S.

(6) Niness, K. R. Inulin and oligofructose: What are they? *J. Nutr., Suppl. 7* **1999**, *129*, 1402S−1406S.

(7) AOAC International. *Official Methods of Analyis of the Association of Official Analytical Chemists*, 15th ed.; The Association of Official Analytical Chemists: Arlington, VA, 1990; Vol. II, Sec. 985.29.

(8) Hoebregs, H. Fructans in foods and food products. Ion-exchange chromatographic method: Collaborative study. *J. Assoc. Off. Anal. Chem. Int.* **1997**, *80*, 1029−1037.

(9) McCleary, B. V.; Blakeney, A. B. Measurement of inulin and oligofructan. *Cereal Foods World* **1999**, *44*, 398−406.

(10) Quigley, M. E.; Hudson, G. J.; Englyst, H. N. Determination of resistant short-chain carbohydrates (nondigestible oligosaccharides) using gas−liquid chromatography. *Food Chem.* **1999**, *65*, 381−390.

(11) Vendrell-Pascuas, S.; Castellote-Bargalló, A. I.; López-Sabater, M. C. Determination of inulin in meat products by high-performance liquid chromatography with refractive index detection. *J. Chromatogr. A* **2000**, *881*, 591−597.

(12) De Leenheer, L.; Hoebregs, H. Progress in the elucidation of the composition of chicory inulin. *Starch/Staerke* **1994**, *46*, 193−196.

(13) Noffsinger, J. B.; Emery, M.; Hoch, D. J.; Dokladalova, J. Liquid chromatographic determination of Polydextrose in food matrixes. *J. Assoc. Off. Anal. Chem.* **1990**, *73*, 51−53.

(14) Quemener, B.; Thibaut, J.-F.and Caussement, P. Determination of inulin and oligofructose in food products, and integration in the AOAC Method for measurement of total dietary fibre. *Lebensm.-Wiss. Technol.* **1994**, *27*, 125−132.

Received for review April 18, 2001. Revised manuscript received July 24, 2001. Accepted July 30, 2001. This work was supported by the University of Buenos Aires (IX 22, 1998−2001).

JF010505O