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Evaluation of fructans in various fresh and stewed fruits by high-performance anion-exchange chromatography with pulsed amperometric detection

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Abstract

Fructans are food-grade non-digestible carbohydrates that exert beneficial nutritional effects. Their characterization and quantification is required for food-labeling purposes. We describe the suitability of high-performance anion-exchange chromatography coupled with pulsed amperometric detection for the identification and quantification of fructans in fresh fruits (various apple and pear cultivars, plum, banana) as well as in commercial stewed fruits obtained from a local manufacturer. After extraction with water and appropriate filtration, inulobiose [β-D-Fru-(2→1)-β-D-fructofuranoside; F2], 1-kestose [β-D-Fru-$(2\rightarrow1)_2$-α-D-glucopyranoside; GF2] and nystose [β-D-Fru-$(2\rightarrow1)_3$-α-D-glucopyranoside; GF3] were completely separated in a single 36-min run using a Dionex CarboPac PA 100 column and the new quadruple-potential waveform, originally tailored for oligosaccharide separation. No measurable amounts of F3 and GF4 were detected within the group of studied fruit products. Peak identification was realized using standards. The method is easy, reproducible, and sensitive since as little as 28 μg of sugar per gram dry matter can be quantified. Banana and plum are the varieties containing the highest levels of fructans (about 6000 μg per gram dry matter). The maturity of the fruit appears to have a great influence on the level of GF2. Samples of apple–banana stewed fruits contained the highest total fructan concentration (about 700 μg per gram dry matter). Accurate quantification of fructans will allow more precise nutritional formulation and diet selection for higher fructan consumption.

Keywords: Fruits; Food analysis; Fructans; Carbohydrates; Inulobiose

1. Introduction

Fructans are widely distributed among prokaryotes, lower and higher plants. These compounds consist of fructose moieties linked to each other by β(2→1) bonds to form inulin, which has a degree of polymerization within the range of 2 to 60 [1]. A glucose moiety may be linked to the end of the chain by an α(1→2) bond, as in sucrose, to form fructooligosaccharides (FOSs) resulting in 1-kestose [β-D-Fru-$(2\rightarrow1)_2$-α-D-glucopyranoside; GF2], nystose [β-D-Fru-$(2\rightarrow1)_3$-α-D-glucopyranoside; GF3] and fructofuranosylnystose [β-D-Fru-$(2\rightarrow1)_4$-α-D-glucopyranoside; GF4] [2]. The daily fructan consumption has been estimated to be 3–11 g in Europe, the most common sources being wheat, onion, banana, garlic and leek [3], while the average daily consumption of FOSs from natural sources is esti-

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mated to be approximately 806 mg/day [4]. Fructans are already bifidogenic at a dose of 2.75 g/day [5] as well as an intake of even 1 g/day of FOSs, although the rate of intestinal bifidobacteria increase was slower as the dose was reduced [6]. Nevertheless, FOS ingestion greater than 44 g/day for men and 49 g/day for women is known to induce diarrhea [7].

The recent use of FOSs as food ingredients has triggered research into their safety and benefits. They have been shown to exhibit beneficial health effects by stimulating the growth of bifidobacteria in the human colon by suppression of putrefactive pathogens [5,6,8]. Because FOSs are not hydrolyzed by enzymes in the small intestine of humans, they reach the colon intact where they are fully metabolized by the colonic microflora [9,10]. The end products of carbohydrate fermentation are gases, namely hydrogen, methane and carbon dioxide [11], lactate, and short-chain fatty acids, such as acetate, propionate and butyrate. The short-chain fatty acids that are produced during fermentation are thought to be readily absorbed by the colonic mucosa. It is known that butyrate serves as a ''fuel'' for the mucosa, whereas acetate and propionate enter the portal blood and may influence systemic carbohydrate and lipid metabolism [12]. Due to their physicochemical properties, sweetening power (0.4 to 0.6 times as sweet as sucrose [4]), and low caloric value (9.5 kJ/g [10], about one-half that of sucrose), FOSs have been added as food components in human and animal diets [4].

Before or during the development of new products, a valid methodology for the determination of fructans and the FOS level must be developed. On the basis of their non-digestibility, it would be logical to consider these compounds as dietary fibers, but they are not measured as such by the Association of Official Analytical Chemists (AOAC) method [13] or by the Englyst et al. method [14] for total dietary fiber determination. Recently, a modification of this method that takes into consideration the total inulin content and that of related oligomers had been proposed [15]. This method includes an enzymatic hydrolysis of the fructose chains followed by D-fructose, D-glucose and sucrose analysis. Although this technique appears to be optimal for inulin, it gives no indication of the concentration of each FOS in the food matrix. Alternatives to this AOAC modified method are high-performance liquid chromatography (HPLC) and gas chromatography (GC). However, these methods suffer from serious limitations in terms of sensitivity, selectivity, ease of use and applicability [16]. In the past few years, much work has been published on the application of high-performance anion-exchange chromatography coupled with pulsed amperometric detection (HPAEC–PAD), which offers a powerful alternative to traditional HPLC methods [17–19]. Most of the HPAEC–PAD applications for the determination of mono-, di-, and oligosaccharides in food products have been performed using a CarboPac PA 1 column, specifically designed by Dionex corporation to separate these saccharides [20]. In previous works we evaluated the applicability of HPAEC–PAD using a CarboPac PA 100 in determining the major carbohydrates found during sugar processing in a local refinery [21–23].

The objective of this study was to determine the suitability of the CarboPac PA 100 column, a pellicular anion-exchange column, as well as the new quadruple-potential waveform developed for enhanced chromatography of oligosaccharides [24], for separating fructans (inulobiose, GF2 and GF3) in a food matrix such as fresh fruit and stewed fruit obtained from a local manufacturer.

2. Experimental

2.1. Chemicals

Standard maltotriose, sucrose, D-glucose and D-fructose were purchased from Sigma (St. Quentin Fallavier, France), while the standard fructooligosaccharide set containing GF2, GF3 and GF4 was from Wako (Neuss, Germany). Raftilose P95 as lyophilized powder was purchased from Orafti (Tienen, Belgium). This mixture was obtained by partial enzymatic hydrolysis of chicory root inulin using endo-inulinase [1]. Sodium acetate (No. 1.06268) was purchased from Merck (Nogent-sur-Marne, France). Sodium hydroxide 46% to 48% (w/w) aqueous solution (S/4930) was from Fisher Scientific (Elancourt, France). Ultra-pure 18 Ω cm deionized water used in the experiments, including that used as HPAEC–PAD solvent, was obtained

from a Maxima Ultra Pure Water system (Elga, Decines, France).

2.2. Sample preparation for analysis by HPAEC–PAD

Fruit samples were obtained fresh from a local retailer or manufacturer (Société de Conserverie Alimentaire Charles Faraud, Monteux, France), while stewed fruits were provided exclusively by the manufacturer. Fruits were peeled, the inedible portion being removed and discarded, while the remaining edible portion (10 g) was cut, lyophilized and weighed for dry matter determination. Stewed fruits (10 g) were also lyophilized.

Lyophilized samples were pounded and rehydrated for 10 min in 50 mL of ultra-pure water. The resulting mixture was filtered through a Whatman No. 1 filter paper, centrifuged and the supernatant passed through a 0.2 μm cut-off microfilter (60372) (Polylabo, Strasbourg, France). Two extractions were realized on each sample, and the extracts were diluted accordingly for chromatographic analysis.

2.3. Conditions used for HPAEC–PAD

Extract samples (10 μL) were injected into a Dionex System DX-500 (Sunnyvale, CA, USA) consisting of a GP 50 gradient pump with on-line degassing and an ED 40 electrochemical detector, operating in the PAD mode, and consisting of an amperometric flow-through cell equipped with a gold working electrode and a silver–silver chloride reference electrode. The pulsed amperometric detector program used for measuring the carbohydrates is shown in Table 1. This system was connected to a personal computer using the Peak Net 5.1 chromatography software program. Products were analyzed using a CarboPac PA 100 (Dionex) anion-exchange column (250×4 mm) equipped with a guard column CarboPac PA 100 (50×4 mm). Gradient elution was applied using two solvents: 0.1 *M* aqueous sodium hydroxide (A) and 0.1 *M* aqueous sodium hydroxide containing 0.5 *M* sodium acetate (B). Total run time per sample was 36 min. The elution began with 100% A for 8 min at a flow-rate of 1 mL/min followed by a linear gradient from 0 to 12% solvent B in solvent A for 22 min and maintained at 12% solvent B for 6 min. The column was then washed for 5 min with 50% solvent B. The next injection was performed after equilibrating the column with 100% eluent A for 19 min. Peak identification was realized using commercial standards. Fructans were quantified by measuring peak areas and comparing them to a standard curve generated by plotting area counts against concentration of standards (0–50 μ*M*). The fructan level (μg/g dry matter) was estimated as follows:

Table 1
Pulsed amperometric cell conditions

Waveform		Integration time	
Time (s)	Potential (V)	Begin (s)	End (s)
0.00	+0.1	0.2	0.40
0.20	+0.1		
0.40	+0.1		
0.41	−2.0		
0.42	−2.0		
0.43	+0.6		
0.44	−0.1		
0.50	−0.1		

$$\text{Fructan}\ (\mu\text{g/g DM}) = \frac{CDVM_r}{1000\text{DM}}$$

where C is the fructan concentration of the extract sample (μ*M*) estimated from the calibration curve, D is the dilution used throughout the experiment, V is the volume of extract (50 mL), M_r is the molecular mass of the compound and DM (g) is the dry matter of the analyzed product. If the fructan content exceeded the linear range of the detector, the solution was diluted accordingly.

3. Results and discussion

The development of analyses for foods and beverages presents a major challenge to analytical chemistry, especially in regard to the determination of polar aliphatic compounds (e.g., carbohydrates). These compounds often have poor optical detection properties and may be present only at trace levels. The HPAEC–PAD method has already been used to rapidly determine the FOS content of a wide variety of foods and feedstuffs using a CarboPac PA 1

column [18,19] and a CarboPac PA 100 [25]. In this study, we determined the potential of the CarboPac PA 100, a column that has never been used to separate inulobiose (F2), GF2 and GF3 by HPAEC–PAD, and to determine their level in fresh fruits as well as, for the first time, in stewed fruits. The proposed method was easy, sensitive and reproducible.

3.1. Pulsed amperometric cell conditions

The new quadruple-potential waveform, described in the Experimental section and recommended by Dionex [26–28], was introduced in this study for the detection of carbohydrates using pulsed amperometry. The new waveform cleans the electrode by application of a potential more negative than the potential limit. In contrast to the commonly used triple-potential waveform [25,29,30], negative cleaning allows the time during which gold oxide is formed to be minimized, thus minimizing the dissolution and resulting recession of the gold working electrode as a result of gold oxide formation/reduction cycles. Preventing gold electrode recession was shown to improve long-term reproducibility [26]. Waveform parameters were chosen so as to maximize the signal-to-noise ratio and freedom from electrode fouling caused by matrix components in the sample. Compared to the triple-potential waveform, the quadruple-potential waveform shows similar minimum detection limits but greatly improved long-term reproducibility [26].

3.2. Analysis reproducibility

When 10 μL of stewed apple ''Fruidoux'' extract was injected five times into the HPAEC–PAD system, the derived concentration of GF2 (mean±standard deviation) was 492±15 μg/g dry matter. Thus, the relative standard deviation [RSD= (standard deviation/mean value)·100] for the analysis was 3%, on average.

3.3. Extraction reproducibility

The extraction method reliability was tested on fresh fruit. Five extractions were realized on the same apple (Golden Delicious) and 10 μL of the extract was injected into the HPAEC–PAD system. The GF2 concentration was 37±2.9 μg/g fresh fruit, representing a RSD of 7.8%. We concluded that the extraction method was reliable and reproducible.

3.4. Percent recovery

Maltotriose solution (50 μL, 100 m*M*) was added to a pounded lyophilized sample in ultra-pure water (50 mL) at the beginning of the extraction procedure. When the extract was analyzed, the maltotriose area was determined (G3 area extract). A control solution consisting of maltotriose solution (50 μL) in 50 mL of ultra-pure water was also analyzed and the maltotriose area determined (G3 area control). Three extractions were realized on two different texture products: a fresh fruit (banana) and a mashed apple–pear. The percent recovery was calculated as

$$\%\ \text{recovery} = \frac{\text{G3 area extract}}{\text{G3 area control}} \cdot 100$$

The recovery was 83±3% for banana and 101±6% for mashed apple–pear. When compared with other data, the present study yielded slightly lower values than those already reported (i.e. 100±10%) [19]. The high recovery of greater than 100% may be attributable to the peak integration sensitivity of the HPAEC–PAD system.

3.5. Evaluation of fructans in various fresh fruits

Fig. 1 shows the HPAEC–PAD chromatogram

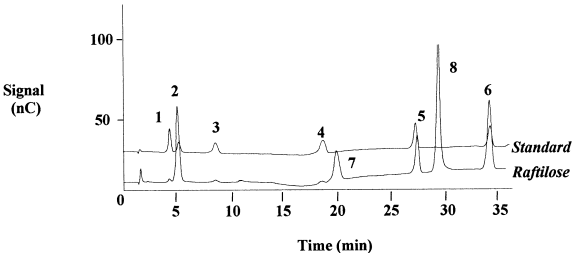

Fig. 1. Chromatogram representing a 10 μL injection of a standard solution containing 20 μ*M* of D-glucose (1; 4.28 min), D-fructose (2; 5.17 min), sucrose (3; 8.68 min), 1-kestose (4; 18.57 min), nystose (5; 27.32 min) and fructofuranosylnystose (6; 34.18 min) as well as of a Raftilose P95 solution (54 mg/mL) containing D-fructose (2; 5.17 min), inulobiose (7; 19.82 min), nystose (5; 27.32 min), inulotriose (8; 29.46 min) and fructofuranosylnystose (6; 34.18 min) (nC, nanoCoulomb).

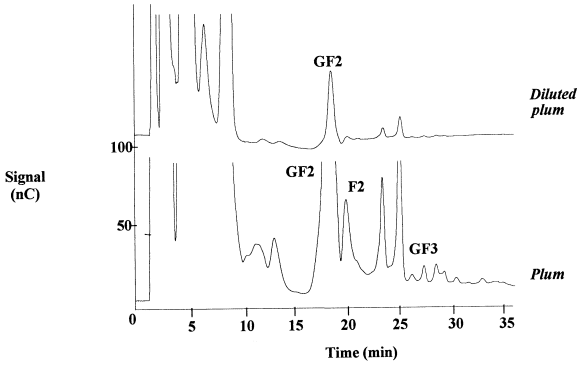

Fig. 2. Fructan profiles of a plum extract diluted (1:10) or undiluted (10 μL injection): 1-kestose (GF2; 18.93 min), nystose (GF3; 27.7 min) and inulobiose (F2; 20.36 min).

obtained with 10 μL of a 20 μ*M* standard calibration mixture containing D-glucose, D-fructose, sucrose, 1-kestose, nystose and fructofuranosylnystose as well as of Raftilose P95 (54 mg/mL) containing D-fructose, inulobiose, nystose and inulotriose (F3). It can be observed that the F2, F3, GF2, GF3, and GF4 chromatogram profiles are in agreement with those reported by other authors using a CarboPac PA 1 column [18,19]. In our study, a good separation of these products was also observed in a single run of 36 min.

Fig. 2 presents the fructan profile of a plum extract diluted (1:10) or undiluted. This fruit was the only sample studied that contained GF3 in addition to GF2 and F2. In this case, dilution (1:10) was necessary to quantify the GF2 level. The elution profiles were similar to standards with respect to retention times. Peak separation was good, allowing all peaks to be quantified.

The fructan compositions of fresh fruits are presented in Table 2. Results are presented as an average on a dry matter basis. Any sample producing sugar unable to be integrated with the sensitivity settings of the HPAEC–PAD is denoted ''nd''. Within the group of fresh fruits studied, chromatogram analysis shows that a large number of fruits contain a mixture of fructans, namely F2, GF2, and GF3. No measurable amounts of F3 and GF4 were detected within the group of studied fruit products. In this work, consequently, total fructan was defined as a combination of F2, GF2, and GF3. It is worth noting that previous studies on fruit sample analyses did not quantify any F2 units [19]. The total fructans determined ranged from 200 to 6660 μg/g dry matter for apple (Granny Smith) and plum d'Agen, respectively. It appears that the fructan level depends on both the variety and the degree of maturity. Indeed, ripe banana (Consul premium) and plum (d'Agen) are the varieties containing the highest levels of fructans, 6020 and 6409 μg/g dry matter, respectively. In the same way, the degree of maturity increases the GF2 level since this value varies from 4323 to 6020 μg/g dry matter, from 236 to 321 μg/g dry matter and from 569 to 1704 μg/g dry

Table 2
Quantification of 1-kestose (GF2), nystose (GF3) and inulobiose (F2) in fresh fruits[a]

Fruit	GF2 (μg/g DM)	GF3 (μg/g DM)	F2 (μg/g DM)	Total fructan (μg/g DM)	Dry matter (%)
Apple, Canada	337±44	nd	107±19	445±38	20±1
Apple, Reinette	298±19	nd	86±6	384±25	20±1
Apple, Granny Smith	161±15	nd	38±6	200±21	16±1
Apple, Golden Delicious	445±48	nd	208±26	655±54	20±1
Pear, Williams green	236±46	nd	315±51	545±59	16±1
Pear, Williams ripe	321±57	nd	300±50	622±90	14±1
Pear, Guyot green	569±60	nd	582±10	1151±50	23±1
Pear, Guyot ripe	1704±297	nd	631±47	2336±328	23±1
Plum, d'Agen	6409±669	47±3	169±21	6660±724	29±3
Banana, Consul premium	4323±337	nd	nd	4323±337	18±2
Banana, ripe Consul premium	6020±863	nd	nd	6020±863	18±1

[a] nd, not detected; DM, dry matter. Each value is the mean of at least four determinations. Total fructan = GF2 + GF3 + F2.

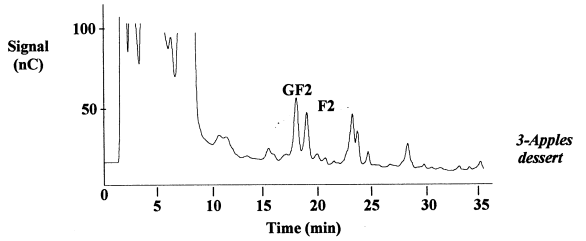

Fig. 3. Fructan profile of a three-apple dessert ''Tante Jeanne'' extract (10 μL injection): 1-kestose (GF2; 18.21 min) and inulobiose (F2; 19.10 min).

matter with the stage of maturity of banana, pear Williams and pear Guyot, respectively. The use of this FOS as a marker of ripeness is now under investigation. Finally, the present study yielded slightly higher values than those reported in the literature for fresh fruits such as banana, apple (Golden Delicious) and pear (Guyot) [19]. This may be attributable to sampling technique as well as sample origin.

It should be noted that the separation of longer fructans, GF_n ($n>4$) and F_n ($n>3$), was not investigated in this work. The decline in detector response for increasing carbohydrate chain length is a well known problem for analyses using HPAEC–PAD equipped with a CarboPac PA 1 [31]. The separation of F2, F3, GF2, GF3, GF4, and other oligosaccharides such as inulo-*n*-ose in a single run is now under investigation using our experimental conditions.

3.6. Evaluation of the fructan level in various processed foods

Fig. 3 shows a good separation of GF2 and F2 in a three-apple dessert ''Tante Jeanne'' extract. Three other sugars, D-glucose, D-fructose and sucrose, were identified but not quantified. Other products were detected in the extract but not identified because of the unavailability of corresponding standards. The compositions of seven stewed fruits, obtained with different fruits and fruit varieties, were determined (Table 3). GF2 and F2 were detected in all samples tested except in the stewed peach, where F2 was absent. The total fructan level of fruit ranged from 299 to 711 μg total fructan/g dry matter for stewed peach ''Fruidoux'' and stewed apple/banana ''Tante Jeanne'', respectively. Among all the stewed fruits studied, the stewed apple–banana product contained the highest level of total fructans (711±24.5 μg/g dry matter). All of these values are inferior to the fructan level determined in fresh fruits.

Furthermore, it is worth noting that our method seems slightly more sensitive than that commonly reported in the literature for similar food matrices, since we were able to quantify a lower sugar level [38 and 28 μg/g dry matter in fresh fruits (Table 2)

Table 3
Quantification of 1-kestose (GF2) and inulobiose (F2) in stewed fruits[a]

Stewed fruit	GF2 (μg/g DM)	F2 (μg/g DM)	Total fructans (μg/g DM)	Dry matter (%)
Three-apple dessert ''Tante Jeanne''	339±12	28±2.5	370±15.0	25±1
Apple dessert ''Carrefour''	283±69	69±8.5	352±30.5	19±1
Stewed apple ''Fruidoux''	510±62	57±5.0	567±67.0	27±1
Stewed apple/blackcurrant ''Fruidoux''	396±3	32±0.5	428±4.0	26±1
Stewed apple/banana ''Tante Jeanne''	660±20	51±4.5	711±24.5	24±1
Mashed pear ''Fruidoux''	228±46	108±7.7	337±52.0	15±2
Stewed peach ''Fruidoux''	299±1	nd	299±1	18±1

[a] nd, not detected; DM, dry matter. Each value is the mean of at least four determinations. Total fructan = GF2 + GF3 + F2.

and stewed fruits (Table 3), respectively] than that usually determined ($\geq$100 μg/g dry matter) [19].

4. Conclusion

HPAEC–PAD using a CarboPac PA 100 column and the new quadruple-potential waveform appears to be a viable means to quantify fructans, namely inulobiose, 1-kestose and nystose, in a single run. The ability to accurately quantify the fructan content of various fruits and processed foods allows the estimation of average fructan intakes. Our results clearly show that fresh fruits could contribute partly to the daily fructan consumption, as well as stewed fruits to a smaller extent, and that the maturity of the fruit appears to be of prime importance in obtaining high fructan levels.

Finally, the food industry is both a pioneer and a benefactor of the technology since demands from customers for detailed information on food content and the desire for high quality control have increased.

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References

[1] L. De Leenheer, in: H. van Bekkum, H. Röper, A.G. Voragen (Eds.), Carbohydrates as Organic Raw Materials, Vol. III, VCH, New York, 1996, p. 67.
[2] D.H. Lewis, New Phytol. 124 (1993) 583.
[3] J. van Loo, P. Coussement, L. de Leenheer, H. Hoebregs, G. Smits, Crit. Rev. Food Sci. Nutr. 35 (1995) 525.
[4] J.E. Spiegel, R. Rose, P. Karabell, V.H. Frankos, D.F. Schmitt, Food Technol. 1 (1994) 85.
[5] M.B. Roberfroid, Br. J. Nutr. 80 (1998) S197.
[6] H. Hidaka, T. Eida, T. Takizawa, T. Tokunaga, Y. Tashiro, Bifidobact. Microflora 5 (1986) 37.
[7] Y. Hata, K. Nakajima, Geriatr. Med. 23 (1985) 1.
[8] H. Tomomatsu, Food Technol. 48 (1994) 61.
[9] M.S. Alles, J.G.A.J. Hautvast, F.M. Nagengast, R. Hartemink, M.K.J. van Laere, J.B.M.J. Jansen, Br. J. Nutr. 76 (1996) 211.
[10] C. Molis, B. Flourié, F. Ouarne, M.F. Gailing, S. Lartigue, A. Guibert, F. Bornet, J.P. Galmiche, Am. J. Clin. Nutr. 64 (1996) 324.
[11] X. Wang, G.R. Gibson, J. Appl. Bacteriol. 75 (1993) 373.
[12] M.S. Alles, N.M. de Roos, J.C. Bakx, E. Van de Lisdonk, P.L. Zock, J.G.A.J. Hautvast, Am. J. Clin. Nutr. 69 (1999) 64.
[13] Association of Official Analytical Chemists, Method 991.43, in: P. Cunniff (Ed.), Official Methods of Analysis, 16th ed., AOAC International, 1995.
[14] H. Englyst, H.S. Wiggins, J.H. Cumming, Analyst 107 (1982) 307.
[15] B. Quemener, J.F. Thibault, P. Coussement, Int. J. Biol. Macromol. 21 (1997) 175.
[16] M. Verzele, G. Simoens, F. Van Damme, Chromatographia 23 (1987) 292.
[17] Y.C. Lee, J. Chromatogr. A 720 (1996) 137.
[18] J.M. Durgnat, C. Martinez, Semin. Food Anal. 2 (1997) 85.
[19] J.M. Campbell, L.L. Bauer, G.C. Fahey Jr., A.J.C.L. Hogarth, B.W. Wolf, D.E. Hunter, J. Agric. Food Chem. 45 (1997) 3076.
[20] Dionex, Better Solutions For Food and Beverage Analysis, 2nd ed., Dionex, Sunnyvale, CA, 1997.
[21] S. Farine, A. Biagini, M.H. Chastan, S. Estoupan, A. Puigserver, Int. Sugar J. 102 (2000) 140.
[22] S. Farine, R. Malgoyre, S. Estoupan, A. Puigserver, A. Biagini, Int. Sugar J. 102 (2000) 182.
[23] S. Farine, R. Malgoyre, S. Estoupan, A. Puigserver, A. Biagini, Int. Sugar J. 102 (2000) 242.
[24] Dionex, Technical Note 20, Sunnyvale, CA, 1993.
[25] C. Corradini, G. Canali, I. Nicoletti, Semin. Food Anal. 2 (1997) 99.
[26] R.D. Rocklin, A.P. Clarke, M. Weitzhandler, Anal. Chem. 70 (1998) 1496.
[27] Dionex, Technical Note 21, Sunnyvale, CA, 1998.
[28] Dionex, Application Update 141, Sunnyvale, CA, 2000.
[29] Dionex, Technical Note 21, Sunnyvale, CA, 1996.
[30] J.M. Campbell, E.A. Flickinger, G.C. Fahey Jr., Semin. Food Anal. 2 (1997) 43.
[31] D.C. Johnson, W.R. Lacourse, in: Z. El Rassi (Ed.), Carbohydrate Analysis, Elsevier, Amsterdam, 1995, p. 391.