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Food Chemistry 69 (2000) 339–344

Food Chemistry

www.elsevier.com/locate/foodchem

Analytical, Nutritional and Clinical Methods Section

Determination of carbohydrates in food samples by capillary electrophoresis with indirect UV detection

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Received 29 August 1999; received in revised form and accepted 31 December 1999

Abstract

An easy and reproducible capillary electrophoretic method with indirect UV detection for the determination of carbohydrates in food samples was described. A highly alkaline pH condition was used in order to charge every carbohydrate negatively. Electroosmotic flow (EOF) was reversed to detect sugar alcohols and to improve the separation of neutral and amino sugars. Under optimized conditions 28 carbohydrates including mono- and disaccharides, uronic acids, sialic acids, amino sugars and sugar alcohols were separated in less than 25 min. The detection limits for fructose, glucose and sucrose were in the range from 12 to 16 mg l^{-1} with pressure injection of 50 mbar for 6 s (ca. 6 nl) at a signal to noise ratio of 3. The calibration curves were linear over the range from 50 to 10 000 mg l^{-1} with correlation coefficients better than 0.9999. This method could be readily applied to the analysis of carbohydrates in several foods and beverages. Sample preparation was minimal and consisted either of dilution or dilution with filtration.

Keywords: Carbohydrates; Foods; Capillary electrophoresis; Analysis

1. Introduction

Carbohydrates such as glucose, fructose, sucrose and some sugar alcohols are widely distributed in various food and beverage, while these compounds are often used as food additives. Therefore monitoring of carbohydrates in food samples is essential and important in the fields of nutrition, biology and food science.

Since carbohydrates are non-volatile compounds, they have been analyzed based on high-performance liquid chromatography (HPLC) methods. The most commonly used analytical technique for carbohydrates in foods is HPLC with refractive index detector (RID). Although HPLC-RID method is simple, it has several demerits. The RID is lack of sensitivity and selectivity. When mobile phase or separation column is changed, the method requires some hours until the baseline is stabilized.

As an alternative technique, HPLC with pulsed amperometric detection (PAD) was introduced, providing lower detection limits of 10 μg l^{-1} for non-derivatized carbohydrates (Rocklin & Pohl, 1983). A large number of HPLC-PAD methods have been developed since then and applied to the analysis of carbohydrates in food samples (Cataldi, Margiotta & Zambonin, 1998;Corradini, Canali & Nicoletti, 1997; Soga, Inoue & Yamaguchi, 1992; Van Riel & Olieman, 1991). However, despite providing high sensitivity analyses with good resolution, HPLC-PAD is not optimally suited for routine analysis. This is because the detector performance depends on the condition of the PAD electrode, which tends to foul through exposure to sample matrix or hydroxides and require a manual polishing procedure. After exchanging the electrode or mobile phase, both triple-step potentials and duration times of the PAD must be optimized. In addition, reproducibility of this technique is not satisfactory with relative standard deviations (RSD) > 10% for peak areas being reported (LaCourse & Johnson, 1991; Weitzhandler, Pohl, Rohrer, Narayanan, Slingsby & Avdalovic, 1996). Both chromatographic methods involve long analysis times, mainly due to lengthy column re-equilibration following the analysis of samples with complex matrices.

Capillary electrophoresis (CE) is a powerful separation technique that can provide high resolution efficiency and is becoming a standard tool for the analysis of many

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0308-8146/00/$ - see front matter
PII: S0308-8146(00)00044-3

compounds. One methodological difference between CE and HPLC is that CE utilizes an open tubular capillary. In CE, even if the sample contains a matrix, it can be injected with minimal sample preparation without decreasing the separation performance. After detection of the peak of interest, the hollow capillary is rapidly flushed with fresh buffer and ready for the next injection. It is therefore a suitable analytical tool for the analysis of food and beverage which can be characterized as samples with various and complex matrices. The only disadvantage of CE is sensitivity, however, it is not a big problem since concentrations of most components in foods are g l^{-1} level.

Regarding carbohydrate analysis, several CE methods have been developed. Since carbohydrates lack both a charge and a strong UV chromophore, several derivatization techniques have been described (Guttman, 1997; Honda, Iwase, Makino & Fujiwara, 1989; Honda, Suzuki, Nitta, Iwase & Kakehi, 1992). While these methods lead to improved sensitivity and resolution, the complexity of derivatization limits its use. Alternatively, methods for the analysis of underivatized carbohydrate have been developed. These methods include the use of high alkaline electrolyte to ionize the carbohydrates and make them suitable for indirect UV detection (Klockow, Paulus, Figueiredo, Amado & Widmer, 1994; Vorndran, Oefner, Scherz & Bonn, 1992). Their techniques enabled to analyze acidic, neutral and amino sugars without derivatization, however, sugar alcohols could not be determined.

Previously the authors (Soga & Heiger, 1998) developed a CE method with indirect UV detection for the composition analysis of monosaccharides in glycoproteins. In this study the method was further developed and applied to the analysis of not only mono and disaccharides but also sugar alcohols in food and beverage samples.

2. Experimental

2.1. Reagents and chemicals

Lactulose, mannuronic acid, mannosamine, *N*-acetylmannosamine and *N*-glycolylneuraminic acid (NGNA) were purchased from Sigma (St. Louis, MO, USA). *N*-acetylglucosamine was from Aldrich (Milwaukee, WI, USA). Xylitol and cetyltrimethylammonium hydroxide (CTAH) solution (25% in methanol) were obtained from Tokyo Kasei (Tokyo, Japan). All other reagents were from Wako (Osaka, Japan). The chemicals used were of analytical or reagent grade. Water was purified with a Milli-Q purification system (Millipore, Bedford, MA, USA).

2.2. Instrumentation

All CE experiments were performed using an Agilent Capillary Electrophoresis System from Agilent Technologies (Waldbronn, Germany). The system comprises a CE unit with built-in diode-array detector and an Agilent CE ChemStation for system control, data collection and data analysis.

2.3. Electrophoretic procedures

Separations were carried out on fused-silica capillaries with 50 μm I.D.×112.5 cm total length (104 cm effective length) or 50 μm I.D.×80.5 cm total length (72 cm effective length). The electrolyte solution was prepared containing 20 mM 2,6-pyridinedicarboxylic acid (PDC) with 0.5 mM cetyltrimethylammonium hydroxide (CTAH) which was used to reverse the direction of the EOF (Tsuda, 1987). The electrolyte pH was adjusted to 12.1 with 1 M NaOH.

Prior to first use, a new capillary was pretreated with the run electrolyte for 20 min. Before each injection, the capillary was preconditioned for 4 min by flushing with the run electrolyte. Sample was injected with a pressure of 50 mbar for 6.0 s. The applied voltage was set at −25 kV and the capillary temperature was thermostatted to 20°C. Detection was carried out with indirect UV monitoring using a diode-array detector. The signal wavelength was set at 350 nm with a bandwidth of 20 nm and the reference was at 275 nm with a bandwidth of 10 nm. This arrangement of signal and reference wavelengths are used so that the negative peaks associated with indirect detection are recorded as positive peaks. A decrease of absorption at 275 nm produced by the presence of carbohydrates is recorded as a relative increase of the signal at 350 nm since the 275 nm signal is used as the reference wavelength.

3. Results and discussion

3.1. Separation of acidic, neutral, and amino sugars and sugar alcohols

In CE ionic species are separated based on their charge and size, therefore the electrolyte pH can be an important factor in optimizing resolution, where it affects an analytes charge. Since pK_a values of neutral sugars and sugar alcohols are high (see Table 1), strongly alkaline condition must be used to ensure ionization. The 20 mM PDC was selected as the background electrolyte (BGE) for this work since it provided excellent capacity for inorganic and organic anions (Soga & Ross, 1999), and carbohydrate analysis (Soga & Heiger, 1998) by indirect UV detection. Separations of carbohydrate were studied with fused-silica capillary 50 μm I.D.×112.5 cm over the pH range from 11.8 to 12.4 with a constant concentration of 20 mM PDC and the better resolution was obtained at pH 12.1.

Also the direction of EOF had significant effect on the carbohydrate determination. Fig. 1 illustrated the

results obtained by normal EOF with positive 25 kV (Fig. 1A) and reversal EOF with negative 25 kV (Fig. 1B). In the normal EOF (Fig. 1A) neutral compounds, e.g. water, were observed as the first large peak and then carbohydrates were detected. The migration times of strongly acidic sugars tended to be long, while neutral and amino sugars migrated early and poorly resolved. In addition, sugar alcohols could not be observed. Although the migration order of carbohydrates was reversed by the reversal EOF condition (Fig. 1B), peak shapes were improved and good resolution was obtained for every component. Furthermore sugar alcohols could be determined in this condition. Therefore, 20 mM PDC and 0.5 mM CTAH, pH 12.1 was chosen as the optimum BGE concentration.

Table 1
Dissociation constants of investigated carbohydrates in water at 25°C

Compound	*pKa*
N-Acetylneuraminic acid (NANA)	2.60[a]
Glucuronic acid	3.20[a]
Galacturonic acid	3.48[a]
Fructose	12.03[b]
Mannose	12.08[b]
Xylose	12.15[b]
Glucose	12.28[b]
Arabinose	12.34[b]
Galactose	12.35[b]
Sucrose	12.51[a]
Raffinose	12.74[b]
Sorbitol	13.6[b]

[a] Zemann, Nguyen and Bonn (1997).
[b] Dean (1992).

Fig. 2 illustrates the separation of the standard mixtures of 28 carbohydrates including monosaccharides, disaccharides, acidic sugars, amino sugars and sugar alcohols. Since the migration times of a few peaks are close, three standard mixtures were prepared and individually determined. Even though some carbohydrates are overlapped or closed at this pH 12.1, they can be separated in a different pH value. For example the fully separation between NGNA and NANA was obtained at a pH > 12.2 (data not shown).

3.2. Linearity and sensitivity

The linearity and sensitivity of the method were tested for fructose, glucose and sucrose, the most common found in food and beverage (Table 2). Despite an increase in peak width for concentrations > 5000 mg l^{-1}, the calibration curves for all analytes were linear over the range from 50 to 10 000 mg l^{-1}. Using eight different concentrations, correlation coefficients were better than 0.9999 for the peak areas. Detection limits for these carbohydrates were between 12 and 16 mg l^{-1} with pressure injection of 50 mbar for 6 s (ca. 6 nl) at a signal-to-noise ratio of three. This wide linearity range and the sensitivity of the method are of great benefit for

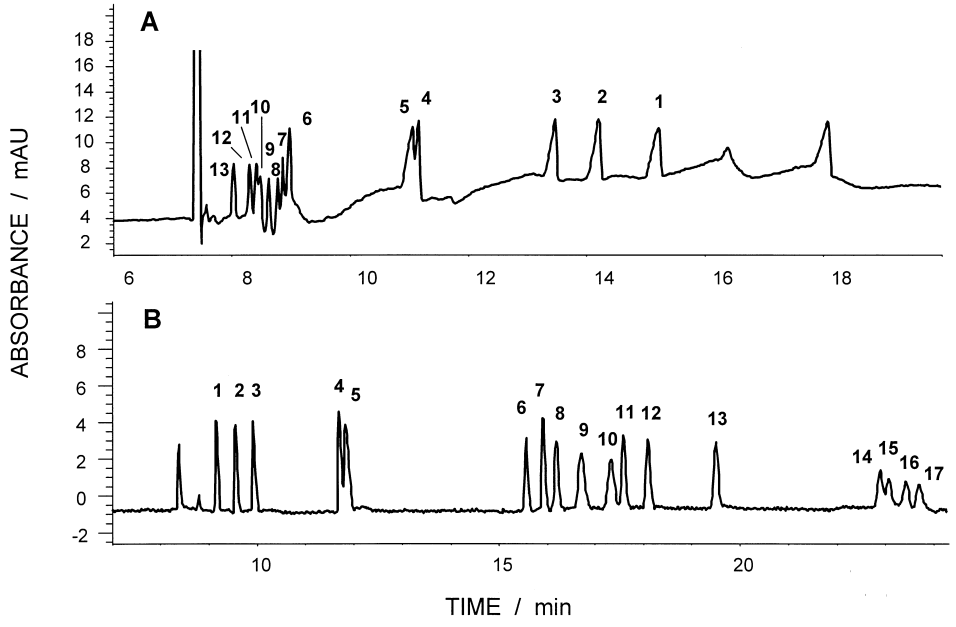

Fig. 1. Comparison of carbohydrate determination obtained by (A) normal EOF with positive 25 kV and (B) reversal EOF with negative 25 kV. Peaks and concentrations: (1) mannuronic acid, (2) glucuronic acid, (3) galacturonic acid, (4) *N*-glycolylneuraminic acid (NGNA), (5) *N*-acetylneuraminic acid (NANA), (6) ribose, (7) mannose, (8) xylose, (9) glucosamine, (10) glucose, (11) galactosamine, (12) galactose, (13) fucose, (14) mannitol, (15) sorbitol, (16) xylitol, (17) inositol, 200 mg l^{-1} each. Experimental conditions: capillary, fused-silica 50 μm i.d. × 112.5 cm (104 cm effective length); background electrolyte, 20 mM PDC, pH 12.1; injection, 6 s at 50 mbar; temperature, 20°C; detection, signal = 350 nm, reference = 275 nm.

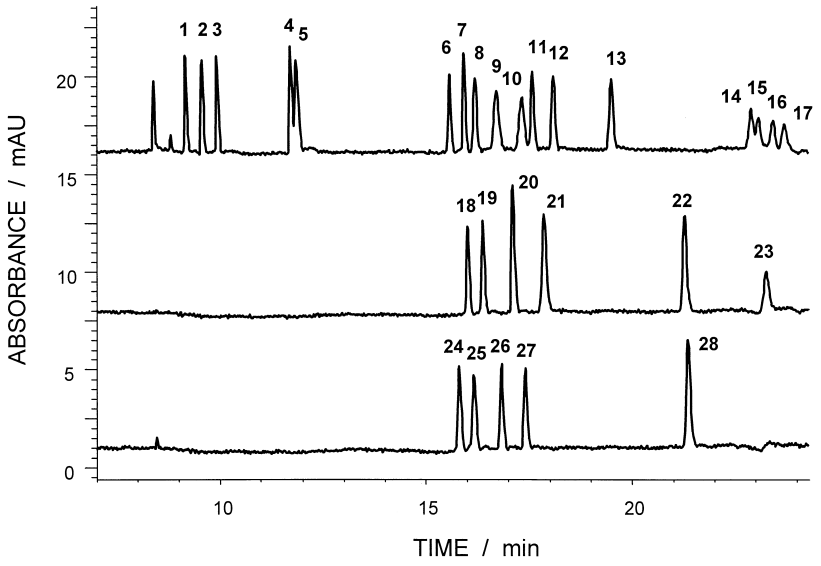

Fig. 2. Separation of 28 carbohydrates by CE. Peak assignment as in Fig. 1, additionally: (18) fructose, (19) rhamnose, (20) lactulose, (21) lactose, (22) sucrose, (23) galactitol, (24) *N*-acetylmannosamine, (25) *N*-acetylglucosamine, (26) *N*-acetylgalactosamine, (27) arabinose and (28) raffinose, 200 mg l^{-1} each. Experimental conditions: capillary, applied potential, −25 kV. Other conditions as in Fig. 1.

Table 2
Linearity and sensitivity

Compound	Linearity correlation	Detection limit (mg l^{-1})
Fructose	1.00000	12
Glucose	0.99990	15
Sucrose	0.99992	16

analyzing food samples, e.g. impurity analysis in sugar product, since both main component and impurity sugars can be analyzed in a single run.

3.3. Analysis of juice, yogurt, pickled ume apricot and sake mash

The method was applied to the determination of carbohydrates in real samples. Since food samples contain only several carbohydrates such as fructose, glucose, lactose and sucrose, a short capillary, total length 80.5 cm, was employed for all subsequent experiments to reduce analysis time. Fig. 3 shows the result obtained from the analysis of an orange juice. In some countries like Japan the tax rates of imported juice depends on the concentration of sucrose. Therefore its analysis is being carried out routinely. After a 1:20 dilution with Milli-Q water the sample was still cloudy, so it was passed through 0.22 μm membrane filter prior to injection. Peaks were identified by their migration times and the concentrations of fructose, glucose and sucrose were calculated as 25, 23 and 43 g l^{-1}, respectively. Satisfactory reproducibilities were obtained with %RSD values

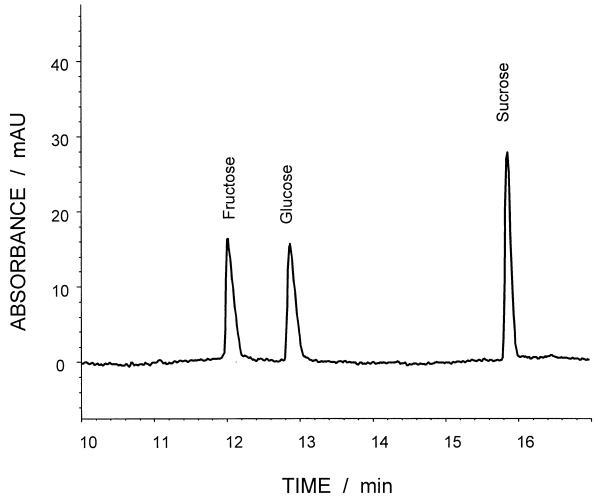

Fig. 3. Electropherogram of carbohydrates in orange juice. Experimental conditions: capillary, fused-silica 50 μm i.d. × 80.5 cm (72 cm effective length); injection, 2 s at 50 mbar. Other conditions as in Fig. 2.

($n = 6$) for migration times between 0.07 and 0.16% and for peak areas better than 1.7%.

Fig. 4 shows an electropherogram of the analysis of carbohydrates in yogurt. Since yogurt contains a large amount of protein, it was initially diluted 1:20 with Milli-Q water and passed through 0.22 μm pore membrane prior to injection. Although lactose and sucrose could be obviously detected with good resolution, migration times of both peaks gradually increased over time; most probably due to protein adhesion to the capillary wall and resulting decrease in the EOF. When using a sample pretreatment involving a 1:20 dilution

followed by centrifugal ultrafiltration through 30 kDa cutoff filter to remove proteins, migration time reproducibility was improved for lactose and sucrose. The %RSD values ($n=6$) for the migration times were 0.06% for both lactose and sucrose and the peak areas were 2.0 and 2.3%, respectively. Proteins removal appears to be mandatory when this method is applied to samples rich in proteins, such as yogurt.

Fig. 5 shows an electropherogram of mono and disaccharides, and sugar alcohols in a pickled ume apricot. Sugar alcohols such as maltitol, sorbitol and xylitol are often added to various kinds of foods as a sweetener, stabilizer, penetrant or keeping humidity agent. Initially the pickled ume apricot was cut up and crushed in a mortar. The mashed apricot was then diluted 1:100 with Milli-Q water. After sonication for 15 min, the solution was passed through 0.45 μm pore membrane and then centrifugally ultrafiltrated through 30 kDa cutoff filter to remove proteins prior to injection. In addition to fructose, glucose and sucrose, maltitol, sorbitol and even ethanol were determined in the same condition.

The method was also applied to the analysis of the carbohydrates in sake mash samples. Measuring the concentrations of carbohydrates during each phase of sake production allows the tracking of the metabolic products of fermentation and the correlation of sake flavor trends. The mono and disaccharide content in the

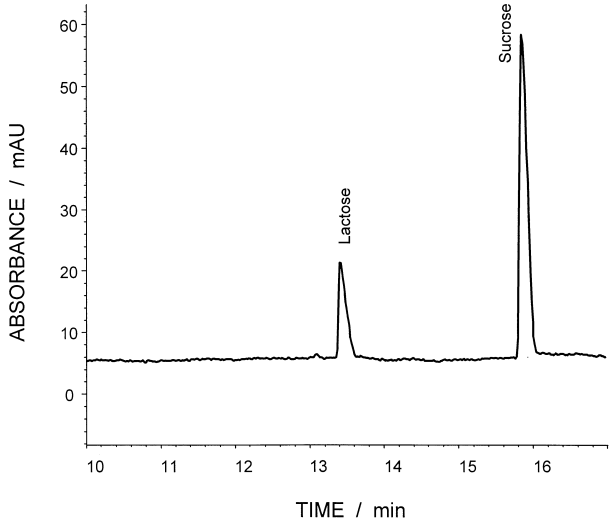

Fig. 4. Electropherogram of carbohydrates in yogurt. Experimental conditions as in Fig. 3.

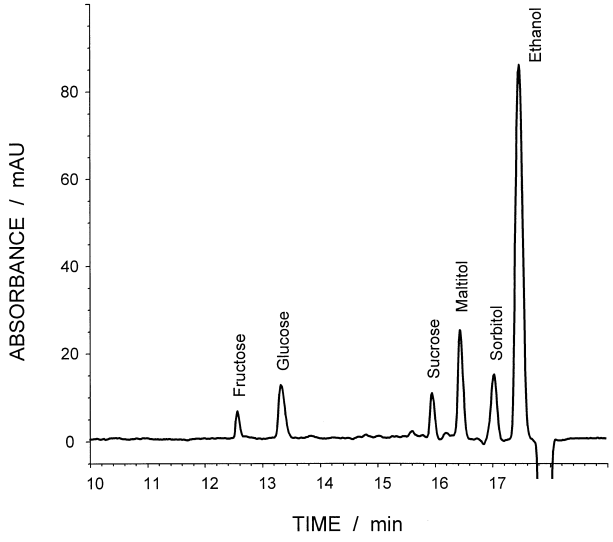

Fig. 5. Electropherogram of carbohydrates in pickled ume apricot. Experimental conditions: injection, 6 s at 50 mbar. Other conditions as in Fig. 3.

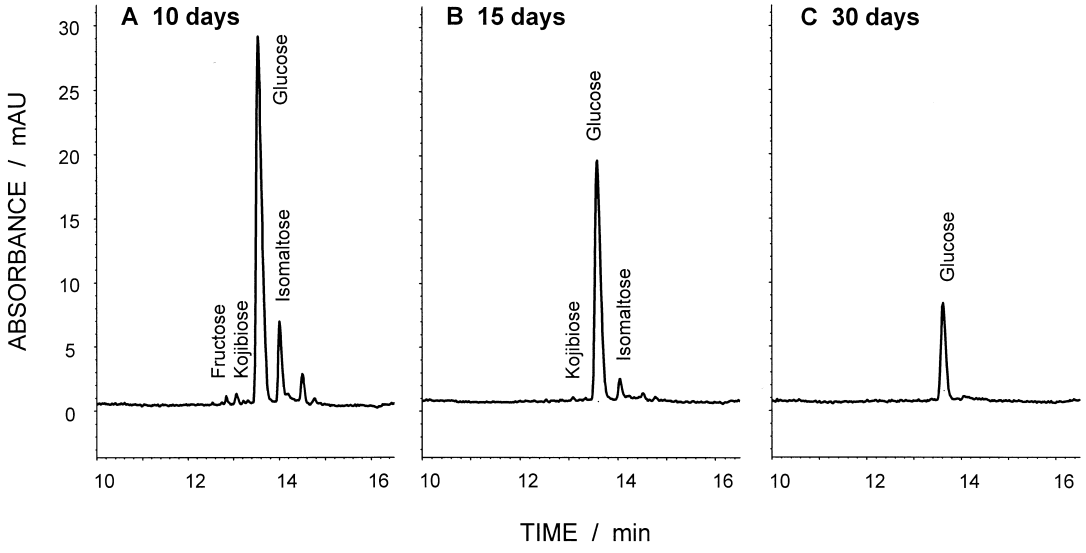

Fig. 6. Comparison with carbohydrates in sake mash after (A) 10 days, (B) 15 days and (C) 30 days of fermentation process. Experimental conditions as in Fig. 5.

sake mash could be monitored during the production process by the described method (Fig. 6). Every sake mash was simply diluted with Mill-Q water (1:40) before injection. After 10 days of fermentation the concentrations of glucose and isomaltose were considerably higher and fructose and kojibiose could also be detected. As the fermentation progressed, the carbohydrate concentration decreased, and after 30 days only a small amount of glucose was determined.

In summary, a simple and reliable CE method with indirect UV detection for the determination of carbohydrates in foods and beverages was described. Compared to other developed techniques, this method has several advantages: (1) most mono and disaccharides, and sugar alcohols can be analyzed without derivatization, (2) a well-defined electropherogram is obtained without other matrix interference, (3) sample preparation is minimum. And it provides excellent reproducibility, good linearity and appropriate sensitivity. Its utility was demonstrated by the analysis of orange juice, yogurt, pickled ume apricot and sake mash. These results indicate that the proposed method can be useful for research and routine analysis of carbohydrates in many kinds of food samples.

Acknowledgements

The authors are very grateful to Akira Kuwada, Fukushima Technology Centre, Koriyama, Japan, for his technical advice and Gordon Ross, Agilent Technologies, Waldbronn, Germany, for critical reading of the manuscript.

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