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A continuous spectrophotometric system for the discrimination/determination of monosaccharides and oligosaccharides in foods

A. Cáceres[1], S. Cárdenas, M. Gallego, M. Valcárcel *

Analytical Chemistry Division, Faculty of Sciences, University of Córdoba, E-14004 Córdoba, Spain

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Abstract

A simple, rapid method for the spectrophotometric discrimination of monosaccharides from the oligosaccharide fraction of fruit juice, jam, syrup and honey samples is proposed. The sample, in alkaline medium, is directly introduced into a flow system and passed through an activated carbon column for its decolourization; then, a volume of 200 μl is injected into the derivatising reagent stream to start the analytical reaction, which takes place at 85°C. The two fractions are discriminated on the basis of the different colours of the derivatives formed; thus, the derivatives of the monosaccharides are yellow while those of the di- and trisaccharides are violet-carmine. The two fractions are monitored at 400 and 540 nm, respectively. Sucrose gives no reaction as it is a non-reducing sugar. The proposed method allows reducing sugars contents from 0.01% to 0.80% w/v to be determined with an average relative standard deviation of 4.5% and a sampling frequency of 10 h^{-1}. The proposed method was validated by applying it to two milk-based and sugar candidate artificial CRMs, with good correlation. The detection limits achieved (0.01% w/v for maltose, lactose and maltotriose at 540 nm) allow adulteration of fruit juices with high fructose syrup from starch at the 4% level to be detected.

Keywords: Continuous system; Discrimination of monossacharides and oligosaccharides; Adulteration of carbohydrate-rich foods

1. Introduction

Ascertaining food authenticity has long been a problem for the food industry. Carbohydrate analysis of foods is an effective method for determining adulteration in honey [1–3], fruit juice [4–7] and syrup [8]. Adulteration in these foods is usually introduced by addition of inexpensive sweeteners such as invert syrups (IS) from cane or beet sucrose and high fructose corn syrup (HFCS), among others [9]. These industrial syrups not only contains the same major carbohydrates present in the sample but also allow their ratios to be adjusted in order to obtain the desired carbohydrate profile. The availability of routine methods for detecting most outstanding type of adulteration decline in its use. However, authentic products exhibit natural variations in these values

* Corresponding author. Tel.: +34-957-218614; fax: +34-957-218606

E-mail address: qalmeobj@lucano.uco.es (M. Valcárcel)

[1] Permanent address: Department of Chemistry, Faculty of Sciences, University of Zulia, Venezuela.

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over ranges that may be wide enough to allow for a modest amount of adulteration to remain undetected. The oligosaccharide profile of the sample is the key to detecting these frauds; for example, maltose and maltotriose are used to control HFCS adulteration [9].

Most available methods for detecting inexpensive sweeteners added to foods rich in carbohydrates are based on the use of high performance liquid chromatography and amperometric detection [3,7,8,9]; gas chromatography [2,4,6] and ^{13}C-isotope ratio mass spectrometry [10,11] have also been used for this purpose, however. Also, site-specific isotopic fractionation with nuclear magnetic resonance determination has been endorsed by AOAC as an official method for the detection of fruit juice adulteration [12]. Alternatives to these expensive choices such as flow injection (FI) based-methods for the determination of the sugar content by spectrophotometric [13–15], chemiluminiscence [16] or amperometric [17] detection have been reported. The spectrophotometric methods, usually requires enzymatic reactions to hydrolyse oligosaccharides to monosaccharides (the latter are often the active molecules in the reaction [18]). Other methods lead to an end product (by degradation of reducing sugars in hot alkaline solutions) with the same absorption properties for all saccharides, which hinders their discrimination [15]. Narinesingh et al. [14] developed an FI method for the spectrophotometric determination of lactose in milk following derivatisation with methylamine; although discrimination between saccharides was not attempted, in a rigorous interference study, the authors found the derivatisation procedure to give yellow derivatives for glucose and galactose. In addition, one other disaccharide (maltose) was found to interfere at similar concentration level and thus to necessitate removal by treatment with maltase prior to analyses. To our knowledge, no references to the continuous discrimination of total monosaccharides from oligosaccharides appears to exist.

The purpose of this work was to develop a simple, rapid spectrophotometric FI method for the discrimination/determination of the monosaccharide and oligosaccharide fractions in carbohydrate-rich foods with a view to establishing the oligosaccharide profile of the sample and facilitate detection of potential adulteration. Monosaccharides and oligosaccharides are discriminated from the different colours of their glycosylamines (yellow and violet, respectively).

2. Experimental section

2.1. Standards and reagents

All reagents were of analytical grade or better. Glucose, galactose, fructose, sucrose, maltose, lactose monohydrate, maltotriose and methylamine hydrochloride were supplied by Sigma-Aldrich Química (Madrid, Spain); all other reagents (sodium hydroxide, acetic acid, sodium acetate, ethanol and activated carbon) were obtained from Merck (Darmstadt, Germany). Two candidate artificial CRMs (351J and 351K, milk powder and sugars), from the European Commission (IRMM) were used for validation.

Stock standard solutions of each carbohydrate at a concentration of 10% w/v were prepared in distilled water/ethanol (95/5, v/v). Working standard solutions were made on a daily basis by appropriate dilution of the stock in 0.25 M NaOH (which remained stable for a working day) and of 0.25% w/v methylamine in 0.25 M NaOH.

2.2. Apparatus

A Hewlett-Packard HP845X UV/VIS diode array spectrophotometer (Palo Alto, CA, USA) controlled by a computer and equipped with a Hellma flow cell (path length 10 mm, inner volume 18 μl, Jamaica, NY, USA) was used. The absorbance was monitored at 400 and 540 nm for monosaccharides and di-/trisaccharides, respectively.

The flow system consisted of a Gilson Minipuls-2, peristaltic pump fitted with poly(vinyl chloride) pumping tubes, a Rheodyne 5041 injection valve, and PTFE tubing of 0.5 mm i.d. for coils and standard connectors. The activated carbon column was constructed by packing a commercially available column of 50 mm × 2 mm i.d. (Omnifit, Cambridge, UK) with 50 mg of the solid. A vaseline-oil bath (Selecta, Barcelona, Spain) was also employed.

2.3. Sample pretreatment

All the samples were purchased at local markets. Following homogenization with a spatula (solids) or by magnetic stirring (slurries), an accurately weighed amount of 1.0 g (solids) or volume of 1.0 ml (juices)

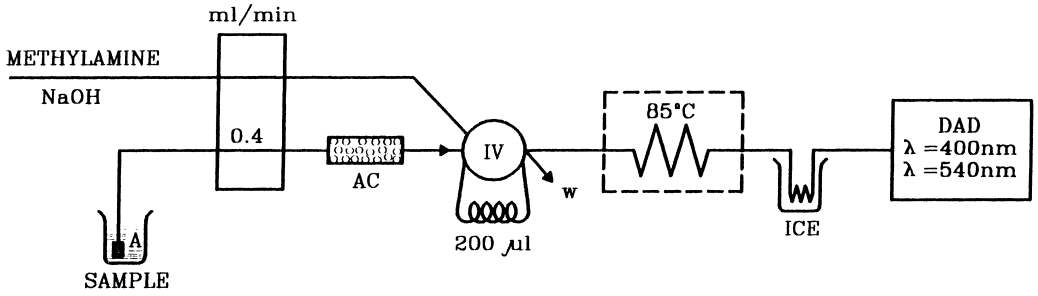


Fig. 1. FI manifold for the spectrophotometric discrimination/determination of monosaccharides from higher saccharides in food samples. A, microfilter; AC, activated carbon column; IV, injection valve; W, waste; DAD, diode array detector.

was diluted to 1/10 with warm distilled water. These dilute samples were rediluted (from 2/10 to 2/100 v/v) with 0.25 M NaOH in order both to condition them to the reaction medium and to accomodate the analyte response within the linear range of the method. These samples can be directly inserted into the flow system. A Nylon membrane (47 mm, 0.45 μm; Sharlau, Barcelona, Spain) microfilter was placed at the entry of the sample aspiration channel to retain any particulate matter if occasionally present in the dilute alkaline samples.

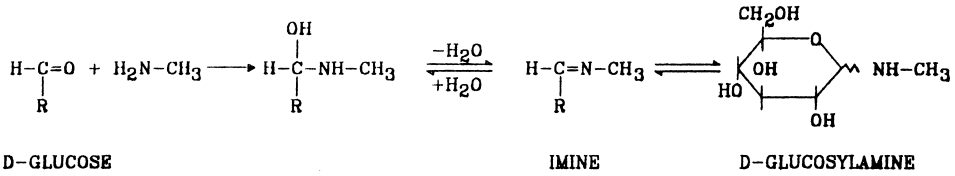
2.4. Procedure

The determination of monosaccharides and oligosaccharides in food samples was carried out in the manifold depicted in Fig. 1. Initially, a standard solution containing variable amounts of the analytes (0.01–0.80% w/v) or a dilute food sample, both in 0.25 M NaOH is continuously aspirated into the manifold and passed through a column packed with ca. 50 mg of activated carbon for its decolourization. A volume of 200 μl of decolourised sample is then injected into a stream of 0.25% w/v methylamine in 0.25 M NaOH at a flow-rate of 0.4 ml/min. A coloured derivative is thus formed in the 5.5 m reaction coil, heated at 85°C by immersion in a vaseline-oil bath, and then cooled in a 1.0 m coil kept at 0°C with ice (to avoid air bubbling). Derivatised analytes are monitored at two different wavelengths (400 and 540 nm) as they pass through the detector. An injection of 200 μl of 0.25 M NaOH is used as blank ($A \cong 0.02$ and 0.01 units at 400 and 540 nm, respectively). Peak height is used as the analytical signal.

3. Results and discussion

3.1. Mechanism of the spectrophotometric method

The mechanism for the derivatisation of sugars with methylamine has not been documented up to date. There are, however, references to the reaction of a reducing sugar with compounds containing an amino group (e.g. free amino acids, peptides, proteins and amines) in foods. This reaction involves the addition of the carbonyl group to a primary amino group, followed by water elimination and leading to an intermediate imine which cyclizes to a glycosylamine (*N*-glycoside) [19]. Similar behaviour can be expected when using methylamine as derivatising reagent, so, the probable mechanism would be as follows (exemplified for glucose):



Based on this mechanism, only reducing sugars can react with methylamine, so, no reaction for sucrose is to be expected as it possesses no free carbonyl group.

From the foregoing, we selected methylamine as derivatising reagent, and monosaccharides, disaccharides and trisaccharides as reactants for discrimination. For this purpose, glucose, galactose, fructose, maltose, lactose, sucrose and maltotriose solutions of variable concentrations in glycine–NaOH buffer at pH 12.7 were mixed in glass vials with the derivatising reagent (0.5% w/v methylamine in 0.02 M sodium sulphite/glycine–NaOH buffer) [14]. The derivatised monosaccharides are yellow in colour and their spectra exhibit an absorption band in the UV region ($\lambda_{max} = 325$ nm) with a shoulder at 400 nm. On the other hand, derivatised disaccharides have a violet-carmine colour and also exhibit absorption bands in the UV region in addition to one nearer to the visible region, (λ_{max} at 540 nm). As expected, sucrose departed from this behaviour and gave no derivative. Maltotriose, the only trisaccharide assayed, also gave a violet-carmine derivative compound, which made its discrimination from the dissacharides virtually impossible.

3.2. Optimisation of the continuous flow method

Initially, a manifold similar to that developed by Narinesingh et al. [14] was used on account of its simplicity. However, as the derivatising reagent contained methylamine, sodium sulphite and glycine–NaOH buffer solution, we initially studied each ingredient separately in order to check whether they were required by the saccharides tested. Thus, the glycine–NaOH buffer (pH 12.7) was replaced with 0.1 M NaOH as it provided similar results. Therefore, aqueous standards of glucose and maltose were prepared at concentrations of 0.2% and 0.3% w/v, respectively, in 0.1 M NaOH, and used in the subsequent monosaccharide and disaccharide test, respectively; the derivatising reagent (0.5% w/v methylamine in 0.02 M sodium sulphite/0.1 M NaOH) was continuously aspirated into an FI system similar to that of Fig. 1and the coloured compounds formed were monitored at 400 and 540 nm for glucose and maltose, respectively. The first experiment was performed to study the effect of the sodium sulfite, a reported stabiliser for derivatised lactose [14]. The effect of its concentration in the derivatising reagent was studied over the range 0–0.5% w/v. The results were different for mono- and disaccharides; thus, the absorbance difference for glucose remained almost constant throughout the studied range, so no advantage or disadvantage was to be expected from its addition to the derivatising reagent; for maltose, signals decreased with increasing amount of sulfite in the reaction medium (e.g. a sulfite concentration of 0.5% decreased the signals by ca. 30%). Therefore, use of sulfite as an ingredient of the derivatising reagent was discarded. The next experiment was focused on the variation of the sample and reagent pH. In order to maintain a constant pH in the reaction plug, this variable was changed simultaneously in both solutions. To this end, the sample and methylamine solutions were prepared in an alkaline medium containing 0.05–0.5 M NaOH. No reaction was observed at NaOH concentrations below 0.1 M; higher concentrations up to 0.3 M favoured the derivatising reaction. However, the stability of the glucose and maltose standards decreased with increasing alkalinity (e.g. standards prepared in 0.5 M NaOH were stable for only 10 min). Therefore, the sample and derivatising reagent were prepared in 0.25 M NaOH for further experiments. The results obtained with the proposed method were compared with reported values for lactose [14]; the signals for glucose and maltose were about 40% than that for lactose. Finally, because the derivatising reagent was a primary amine, various alkylamines (viz. methyl-, ethyl- and propylamine) were tested, all dissolved in 0.25 M NaOH. By using the flow system of Fig. 1, glucose and maltose reacted only with methylamine. This was experimentally checked by adding solutions containing the different amines at a 0.25% w/v concentration each in an alkaline medium to glass vials holding standards of glucose and maltose in the same medium. Following mixing and heating at 80°C, the development of the derivatisation reaction was confirmed by the yellow or violet-carmine colour observed. The reaction time increased with increasing molecular weight in the amine. The reaction was virtually complete within a few minutes for glucose and maltose with methylamine; the other amines required longer times (ca. 1 h). This can be ascribed to the stearic hindrance of the ethyl and propyl groups relative to the methyl substituent, which is directly

related to the kinetics of glycosylamine formation. Methylamine was thus selected and tested at concentrations over the range 0.05–0.5% w/v; the absorbance difference at 400 and 540 nm increased with increasing the concentration of the amine up to 0.2%, above which it remained virtually constant. A concentration of 0.25% of methylamine in 0.25 M NaOH was thus selected for the derivatising reagent.

As in the lactose method [14] the temperature was found to have a decisive influence on the derivatisation reaction, its effect was studied over the range 40–90°C by immersing the reaction coil into a vaseline-oil bath. Glucose and maltose required heating at least at 80°C to ensure full colour development. A temperature of 85°C was thus selected; however, because high temperatures resulted in the formation of bubbles that disturbed the analytical signals, a cooling reactor of 1.0 m was inserted before the detection system.

The influence of the flow variables affecting the formation of the coloured compound, viz., the length of the reaction coil and the reagent flow-rate, was investigated. The length of the reaction coil was found to scarcely influence the reaction of maltose, in fact, signals remained almost constant at lengths over 3.5 m. The effect was more marked for glucose; signals increased up to a length of 5 m, beyond which they remained virtually constant. The influence of the flow-rate of the reagent, which acted as carrier of the injected sample, was studied over the range 0.2–1.0 ml/min; in general low flow-rates resulted in increased absorbance differences for maltose and, especially, glucose. This can be ascribed to the residence time increasing with decreasing flow-rates, which consistent with the longer reaction coil required for glucose as result of the lower reaction rate of this monosaccharide. With a residence time of 2.5 min, derivatisation within the plug was virtually complete, so the response obtained on passage of the coloured plug through the detector was maximal. This time was therefore, chosen as optimal and the flow-rate and reaction coil length set at 0.4 ml/min and 5.5 m, respectively, to obtain it. Finally, the injected sample volume was studied over the range 50–500 μl. The absorbance difference increased with increasing injected sample volume up to 175 μl and remained virtually constant up to 300 μl, above which a double signal was obtained as a result of incomplete homogenization of the plug leading to the formation of two reaction zones. A volume of 200 μl was selected in order to ensure a single signal.

The UV–Visible absorption spectra for the derivatised carbohydrates studied are shown in Fig. 2. As can be seen, glucose can be determined at $\lambda = 400$ nm; however, fructose exhibits a similar absorption spectrum. On the other hand, maltose can be determined at $\lambda = 540$ nm, but lactose and maltotriose also absorbed at this wavelength; this spectral behaviour precludes the discrimination between these compounds. Therefore, the proposed method was re-aimed at discriminating between the total monosaccharide and oligosaccharide fractions in carbohydrate-rich foods.

3.3. Calibration, sensitivity and precision

Under the selected chemical and flow conditions, the manifold depicted in Fig. 1 was used to run calibration graphs to determine glucose, galactose, fructose, maltose, lactose and maltotriose. The graphs were constructed by using 200 μl of standard solutions containing variable concentrations of the analytes between 0.01% and 0.80 % w/v in 0.25 M NaOH with three replicates per standard and seven standards per calibration graph; absorbance differences were obtained versus a blank (200 μl 0.25 M NaOH). The results are listed in Table 1. As can be seen, non-linear relations were obtained for monosaccharides (glucose, galactose and fructose) that conformed to second-order polynomial equations with good correlation coefficients ($r^2 \geq 0.994$). This non-linearity indicates that the reaction does not take place at low monosaccharide concentration. In order to discriminate between mono- and oligosaccharides, two calibration graphs were constructed using maltose, lactose and maltotriose standards at the previous two wavelengths (400 and 540 nm). Excellent linearity ($r^2 \geq 0.996$) was obtained in all instances. The detection limits of Table 1 were calculated as three times the standard deviations of the absorbances obtained for 20 blanks (200 μl 0.25 M NaOH) measured at 400 and 540 nm. The precision (repeatability) of the method expressed as the relative standard deviation, was checked on 11 individual samples containing concentrations of the analytes in the middle portions of the calibration graphs. The most interesting conclusions that can be

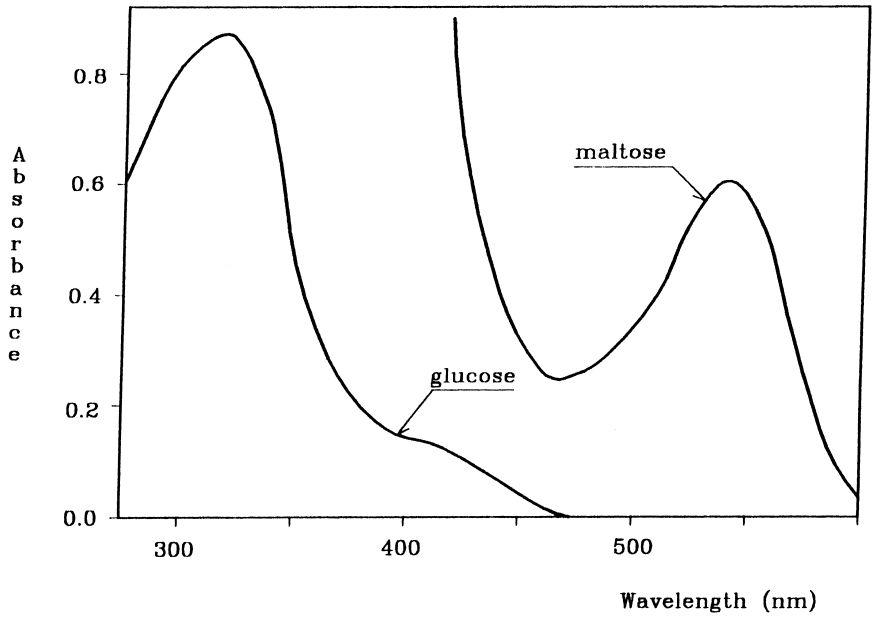


Fig. 2. Absorption spectra for glucose (0.10% w/v) and maltose (0.5% w/v) derivatives, obtained under optimal flow conditions (for details, see text).

Table 1
Characteristic parameters of the calibration graphs and analytical figures of merit for the determination of carbohydrates

Compound	Wavelength (nm)	Detection limit (%)	Regression equation[a]	Linear range (%)	RSD (%)
Glucose	400	0.02	$Y = 6.60\ X^2 + 0.50\ X + 0.02$	0.05–0.40	4.7
Galactose	400	0.02	$Y = 9.09\ X^2 + 0.50\ X + 0.02$	0.05–0.35	4.5
Fructose	400	0.02	$Y = 20.30\ X^2 + 0.18\ X + 0.01$	0.07–0.25	4.3
Maltose	400	0.003	$Y = 7.35\ X + 0.02$	0.01–0.17	4.8
	540	0.01	$Y = 1.25\ X + 0.01$	0.04–0.80	3.7
Lactose	400	0.003	$Y = 7.44\ X + 0.02$	0.01–0.17	5.0
	540	0.01	$Y = 1.33\ X + 0.01$	0.04–0.80	3.8
Maltotriose	400	0.003	$Y = 7.26\ X + 0.02$	0.01–0.17	5.2
	540	0.01	$Y = 1.20\ X + 0.01$	0.04–0.80	3.9

[a] Y: absorbance difference, X: analyte concentration (%).

drawn from Table 1 are as follows: first, the detection limit at 400 nm is lower for oligosaccharides than for monosaccharides; second, as expected, the sensitivity for oligosaccharides is higher at 400 nm than that at 540 nm; third, maltose, lactose and maltotriose can be determined by using the same calibration graph, taking into account the similarity of the slopes of the calibration graphs (mean values 7.35 and 1.26 at 400 and 540 nm, respectively). Finally, the precision of the method is similar for all saccharides.

3.4. Validation of the method

As no reference materials with certified oligosaccharide contents were available; two candidate artificial milk powder and sugar CRMs with uncertified values were used for validation. The first sample, 351J, contained uncertified amounts of fructose, sucrose and maltose; its lactose concentration was determined by HPLC with refractive index detection in our laboratory. The second one, 351K, had uncer-

tified amounts of sucrose; its lactose and maltotriose concentrations were determined by HPLC as above. Table 2 lists the uncertified and HPLC values for these samples. The proposed method was validated with both candidate artificial CRMs after appropriate treatment. To this end, an accurately weighed amount of 2.5 g of the milk powder was placed in a glass vessel and dissolved in 15 ml of distilled water. After homogenization with magnetic stirring, the solution was precipitated with 1 ml (351J) or 0.5 ml (351K) of 0.1 M acetic acid/acetate buffer at pH 4.5 [19]. Precipitation was instantaneous and the mixtures were centrifugated for 5 min and filtered. The supernatants and washing aqueous solutions were diluted to 25 ml with distilled water in calibrated flasks. For the determination of sugars, 0.3–0.5 ml of the solution was diluted to 5 ml with 0.25 M NaOH in a calibrated flask. Each sample was treated in triplicate and analysed five times at different dilution levels to obtain a mean value and its standard deviation ($n = 15$). The results obtained for all saccharides are listed in Table 2. As can be seen, the fructose content can be directly obtained with the proposed method by subtracting the oligosaccharide contribution at 400 nm (sucrose does not react under these conditions), based on their differential sensitivity at 400 and 540 nm. The oligosaccharide concentrations found are directly proportional to the combined contents of the individual compounds (e.g. maltose + lactose = 14.4%, for 351J).

Table 2
Analysis of candidate artificial milk powder and sugar CRMs with the proposed method

Sample	Saccharide	Uncertified value (%)	FI method (%)
351J	Fructose	15	14.0 ± 0.7
	Sucrose	10	nd
	Maltose	5	13.7 ± 0.8
	Lactose	9.4 ± 0.4[a]	
351K	Sucrose	10	nd
	Lactose	28 ± 1[a]	31 ± 2
	Maltotriose	1.5 ± 0.1[a]	

[a] Concentrations ± standard deviations found by HPLC-IR, nd: not detected.

3.5. Discrimination and determination of mono- and oligosaccharides in food samples

The influence of other reductants such as ascorbic acid, frequently present in food samples as a natural or spiked constituent was studied. For this purpose, individual solutions of all the saccharides were prepared at concentrations falling in the middle of the linear range (see Table 1) in 0.25 M NaOH to which ascorbic acid was added as potential interferent; the samples were processed as described under Procedure (Section 2.4). Taking into account the low concentration of ascorbic acid (0.01–0.9 g/l in fruit juice) relative to sugars in food [19], the maximum concentration tested was 1% w/v. The results in its presence and absence were consistent, which reveals that the acid does not interfere. In order to avoid the potential interference of natural/synthetic colouring in processed samples, the decolourization of the dilute sample was necessary. Therefore, a column, packed with 50 mg of activated carbon was inserted before the injection valve (see Fig. 1). The adsorption capacity of the sorbent was studied by using a yellow food (orange squash) and a red one (strawberry jam) as test sample. Both samples were diluted 1:50 as described in Section 2.3; 1 ml of dilute sample in an alkaline medium (an excess sample volume was used to fill the sample loop) was passed through the activated carbon column, the effluent being continuously monitored at 400 and 540 nm. Both effluents were colourless and their signals negligible; a column packed with 50 mg of sorbent was therefore, adopted to decolourise the food samples. Finally, in order to ensure that no analytes would be adsorbed on the column during the decolourization step, individual standards of the six analytes tested were prepared at a concentration of 0.2% in 0.25 M NaOH and inserted into the flow system containing the activated carbon column; the signals thus obtained were quite consistent with those from which the calibration curves were constructed.

Following pretreatment (Section 2.3) the dilute samples were introduced into the flow system and analysed. The results obtained are given in Table 3. Average concentrations were calculated from five individual amounts of each sample and determinations were all done in triplicate ($n = 15$). The seven fruit juices samples were found to contain monosaccharides only, so no adulteration with industrial syrups was apparent. The monosaccharide content in orange squash was lower than those in the commercial fruit juices analysed; this indicates that, during industrial

Table 3
Analysis of sugars in food samples[a]

Food (fruit juices)	Monosaccharides (as glucose), g/l	Oligosaccharides (as maltose), g/l	Food (jams)	Monosaccharides (as glucose), (%)	Oligosaccharides (as maltose), (%)
Orange squash	62 ± 3	nd	Apricot 1	29 ± 2	nd
Apple	96 ± 5	nd	Apricot 2	32 ± 2	nd
Peach-apple	110 ± 6	nd	Plum	30 ± 1	nd
Pinneapple	121 ± 6	nd	Strawberry	48 ± 2	nd
Grapefruit	141 ± 6	nd	Peach	50 ± 2	nd
Peach-grapefruit	116 ± 6	nd	Orange	52 ± 3	nd
Pinneapple-grapefruit	124 ± 6	nd	Peach, light	52 ± 3	0.5 ± 0.1[b]
Tropical fruits	109 ± 6	nd	Pinneapple	56 ± 3	nd
			Rasbperry	60 ± 4	nd
Corn syrup	17 ± 1[c]	19 ± 1[c]			
Caramel syrup	21 ± 1[c]	17 ± 1[c]			
Honey	65 ± 3[c]	2.2 ± 0.1[c]			

[a] Average of the individual determination ± standard deviation; nd: not detected at a sample dilution 1/10.
[b] Sample diluted ten times.
[c] Results expressed as % w/w.

proccessing, the juices are usually spiked with sugars (normally glucose, fructose and sucrose), which is quite legal. On the other hand, the lowest monosaccharides contents among the commercial fruit juices were found in the apple juice and the highest in the grapefruit one, consistent with reported data [19,20].

The sugar content in jam is higher than that in fruit juice because the former is obtained by boiling a fruit concentrate with sugars. The apricot and plum jams contained the lowest concentrations of monosaccharides, while raspberry and pinneapple jams exhibited the highest. It is worth noting the presence of oligosaccharides in the peach light jam sample; however, the signal obtained at the dilution level used (1/50) only allowed qualitative detection. In order to quantify its oligosaccharide content, the lowest dilution described in Section 2.3 (1/10) was tested, the pH being adjusted with few microliters of concentrated NaOH. The presence of oligosaccharides in this sample can be ascribed to the use of glucose syrup during industrial processing. As no information about individual saccharides is provided by the manufacturer, their signal can be ascribed to potential adulteration; however, confirming this assumption would have entailed the individual identification of each oligosaccharide by HPLC technique so as to identify its origin. Finally, oligosaccharides were easily determined in syrups and honey samples.

In order to confirm the ability of the proposed method to discriminate between monosaccharides and oligosaccharides, three fruit juices and three jams containing variable amounts of monosaccharides were spiked with maltose at variable concentrations, 3–10 g per liter (juices) or kg (jams) and allowed to stand refrigerated overnight. The spiked samples were then diluted 1/50 before analysis. Recoveries ranged between 95.5 and 98.9% for fruit juices and from 96.0 to 100.7% for jams.

4. Conclusions

The proposed method can be used to discriminate between the monosaccharide and disaccharide fractions in carbohydrate-rich foods with a view to detecting adulteration without the need for sophisticated, expensive instrumentation or skilled operators. Chromatographic techniques can be used for confirmation here. The proposed automatic flow system provides the intrinsic advantages of continuous methods (viz. low sample and reagent consumption, high throughput) with minimal sample manipulation as no interferences from major or minor components of foods are apparent. Future experiments could be aimed at extending its application to other matrices such as dairy products and to the detection of both reducing and non-reducing sugars hydrolysable to reducing saccharides by insertion of a solid-phase catalytic reactor.

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