Analytical Methods

Simultaneous determination of monosaccharides and oligosaccharides in dates using liquid chromatography–electrospray ionization mass spectrometry

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ABSTRACT

Ultra performance liquid chromatography coupled to mass spectrometry was used for the simultaneous separation and determination of reducing monosaccharides (fructose and glucose), a non-reducing disaccharide (sucrose) and oligosaccharides (kestose and nystose) in HILIC mode. The chromatographic separation of all saccharides was performed on a BEH amide column using an acetonitrile–water gradient elution. The detection was carried out using selected ion recording (SIR) acquisition mode. The validation of the proposed method showed that the limit of detection and limit of quantification values for the five analyzed compounds were in the range of 0.25–0.69 μg/mL and 0.82–3.58 μg/mL, respectively; while the response was linear in the range of 1–50 μg/mL. The developed method showed potential usefulness for a rapid and sensitive analysis of underivatized saccharides and was used for determination of sugars in three date samples (Sefri, Mabroom, Ghassab) which were soxhlet extracted by ethanol.

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1. Introduction

Saccharides are the most abundant compounds found in nature (Peris, 1996) and are considered as a common source of energy in living organisms (Westman, 2002). In the recent years, saccharides have attracted much attention of researchers, because they are used as functional ingredients, besides their traditional use as an energy source and sweetener, with a great potential to improve food quality (Bekers, Marauska, Grube, Karklina, & Duma, 2004). Among the saccharides, fructo-oligosaccharides (FOSs) represent a major class of fructan oligomers; they are considered to be a promising functional food (Goulas, Kapasakalidis, Sinclair, Rastass, & Grandison, 2002; Li, Li, Shen, & Chen, 2002). FOSs such as kestose are very intensively studied for their potential use as natural low-energetic sweeteners and additives in various foods and syrups (Wang, Zhang, & Ouyang, 2002). FOSs are used not only as energy reserves but additionally as osmoregulators due to their solubility in water (Vågen & Slimestad, 2008). They improve the intestinal flora and increase calcium and magnesium absorption (Ohta et al., 1995). Owing to their relatively high sweetness,

noncariogenicity and nondigestibility, they have broad applications in diabetic, child and low-energy foods (Mabel, Sangeetha, Platel, Srinivasan, & Prapulla, 2008). Moreover, they stimulate absorption of magnesium and calcium, and decrease the total cholesterol, phospholipids and triglycerides in serum (Raschka & Daniel, 2005; Sakai, Ohta, Takasaki, & Tokunaga, 2000). FOSs have been widely used in bio-industries as sweeteners because of their favorable functional properties (Cheng & Lee, 1992).

A simultaneous and rapid analysis of mono- and oligosaccharides in foods would be useful for manufacturers' quality control and improvement of their products, as well as inspection by public agencies. With the increased interest in the possible link between FOSs intake from functional foods and health, the need for reliable data on the individual FOSs content of those foods has become very important.

The separations of underivatized sugars have continued to gain attention in recent years due to their ubiquity in nature and their significant roles and uses in industry (Varki, 1993). Native sugars could not be retained on alkyl-bonded silica columns such as C_{18} owing to their high hydrophilicity, and thus their separation is not possible using reversed-phase liquid chromatography. On the other hand, a variety of chromatographic methods have been proposed to separate saccharides and oligosaccharides. They include ligand-exchange chromatography (Stefansson & Westerlund,

1996), size exclusion chromatography (Churms, 1996; Huisman et al., 2001), and widely used hydrophilic chromatography on silica-based bonded polar stationary phases (Alpert et al., 1994; Lin & Lee, 1998). The separations of underivatized carbohydrates require specific types of columns such as alkylated silica-bonded phases (Brons & Olieman, 1983; Klein, Carnoy, Lo-Guidice, Lamblin & Roussel, 1992), amino-bonded stationary phases (Blanken, Bergh, Koppen, & Van den Eijnden, 1985; Nikolov & Reilly, 1985; Rabel, Caputo, & Butts, 1976) or various ion-exchange media (Liu, Urgaonkar, Verkade, & Daniel, 2005; Sakr, Abu Zeid, Hassan, Baz, & Hassan, 2010) in which specific counter-ions are added to affect the separation.

However, most of the columns have problems, particularly in terms of column stability, lifetime and separation reproducibility. Therefore, a UPLC amide BEH column was used in this work to separate mono and oligosaccharides without prior derivatization. The amide-based stationary phases have shown a high chemical stability, a wide pH range, a good efficiency and reproducibility; they allow separation of a wide range of highly polar compounds (Liu et al., 2005). The aim of the present work was to develop a reliable method for separation of a mixture of saccharides containing glucose, fructose, sucrose, kestose and nystose by ultra-performance liquid chromatography hyphenated to tandem mass spectrometry (UPLC–MS) using an electrospray ionization source. The proposed method has shown potential usefulness for the sensitive and quick analysis of underivatized individual saccharides from biological matrices like date extracts.

2. Materials and methods

2.1. Chemicals and reagent

The standards sucrose, D-glucose, D-fructose and sucrose were purchased from Sigma (St. Quentin Fallavier, France), while the FOSs set containing kestose, and nystose was obtained from Wako (Neuss, Germany). Acetonitrile and ethanol 99.8% were purchased from BDH Chemicals Ltd. (Poole, England). Ammonia for mobile phase preparation and lead acetate were obtained from Panreac (Barcelona, Spain). Water was purified through a Milli-Q water purification system (Millipore Corporation, Bedford, MA, USA). A stock standard solution of saccharides 100 µg mL^{-1} was prepared in mobile phase (acetonitrile/water, 50:50, v/v). Standard mixtures of saccharides at different concentration levels (1–50 µg/mL) were prepared for calibration curves. All standards and samples were filtered through a 0.22 µm PVDF syringe filter (Membrane Solutions, TX, USA) before being injected into the UPLC–MS system. All solvents used were of HPLC grade.

2.2. Ultra performance liquid chromatography

The chromatographic separations of the investigated saccharides standards and samples were carried out on a Waters Acquity ultra-performance liquid chromatography system (Waters, Manchester, UK). This UPLC instrument consisted of an Acquity UPLC binary solvent manager, a sample manager and a column heater equipped with a BEH amide column (50 mm × 2.1 mm i.d., 1.7 µm particle size) (Waters, Milford, MA, USA). Optimum separation of all analytes was achieved using a mixture of acetonitrile and water with 0.1% ammonia, according to the gradient mobile phase described in Table 1. The injected volume of sample was 1 µL.

2.3. Mass spectrometry

The ultra-performance liquid chromatography system was coupled to a tandem quadrupole mass spectrometer detector (TQ Detector) from Micromass (Milford, MA, USA) equipped with an

Table 1
Detailed gradient flow profile for UPLC system.

Time (min)	ACN:Milli Q with 0.1% NH$_3$ (%)	Flow (mL/min)	Curve
Initial	95:5	0.40	6
2	95:5	0.40	6
4	70:30	0.40	6
6	60:40	0.40	6

electrospray ionization (ESI) source. An Oerlikon rotary pump, model Sogevac SV40 BI (Bourg-Lès-Valence, France) was used to provide the primary vacuum to the mass spectrometer. The ionization source was operated in the negative mode and the data was acquired in single ion reaction (SIR) monitoring considering the deprotonated molecular ion of each compound as a precursor ion. Source working conditions were as follows: cone voltage: 60 V; capillary voltage: 3.0 kV; source temperature: 120 °C; desolvation temperature: 300 °C; cone gas flow rate: 60 L/h; desolvation gas flow rate: 600 L/h. Nitrogen (99.99% purity) was used as cone and nebulizing gas and produced with a Peak Scientific nitrogen generator (model NM30LA, Inchinann, UK). Data acquisition and processing were carried out using MassLynx V4.1 software.

2.4. Sample collection and identification

The date palm fruit samples were collected at the "Tamr stage" (full ripeness). They included three types of sun-dried dates (DD) such as, Mabroom (elongated, medium to large, light brown), Red Bodara also known as Ghassab (ovoid elongated, medium to large, dark red), and one type of semi-dry dates (SDD), namely Sefri (cylindrical, medium, reddish brown). The commercial way of drying the date samples was at 30–50 °C for 7–10 days which allows easy preservation of the samples. All date varieties were procured from retail local markets of cultivars grown in Besha region, Saudi Arabia, at the end of the 2011 harvest season. Identification and description of each cultivar was visually verified by the experienced farmers and according to previously published papers (Al-Abdoulhadi et al., 2011; Sakr, Abu Zeid, Hassan, Baz & Hassan, 2010). In order to avoid any confounding differences in the environmental factors-such as type of soil and fertilizers, irrigation system and humidity that may affect the chemical composition of the dates, the samples were collected from different local markets and pooled together. The study was conducted on sun-dried and semi-dry dates due to their higher consumption compared with fresh dates.

2.5. Sample preparation

Mature fruits of uniform size, free of physical damage and injury from insects or fungal infection, were selected and used for all experiments. After removing the seeds, the date fleshes were rinsed under running tap water, and finally with distilled water, plotted dried with tissue paper, then dried for 24–72 h at 40–60 °C under sunlight. The dried samples were then powdered using a mini-grinder (SM-2460CG-M, Sanyo, Japan) and stored using airtight containers in a freezer at −20 °C prior to extraction and analysis.

2.6. Extraction procedure

The extraction of sugars in date flesh was carried out by following previously described methods (Ali, Al-Kindi, & Al-Said, 2009; Kafkas, Koşar, Tüemiş, & Başer, 2006; Macrae, 1988; Myhara, Karkalas, & Taylor, 1999; Sahari, Barzegar, & Redfar, 2007) with

minor modifications. Samples of dates (2.5 g) were first extracted by refluxing twice with 50 mL ethanol (85%). In this step the extract was placed in a round-bottom flask (250 mL) connected to a glass condenser and magnetically stirred on a water bath (80 °C) for 30 min. This step was repeated twice, to dissolve all the sugars. The extract was filtered though paper filter (Whatman, No. 2) and the residues were washed with ethanol (75%). 1.5 g of lead acetate was added with gentle heating in water bath at 40 °C for 15 min to precipitate the proteins. After centrifugation for 30 min at 4000 rpm, the supernatants were evaporated to dryness in water bath at 70 °C under reduced pressure. The residues were dissolved in a mixture of acetonitrile 98% and Milli Q water (1:1, v/v%) in a volumetric flask (50 mL). Note: as sugars do not dissolve in acetonitrile, they were first dissolved in water and then diluted to the mark with an equivalent volume of acetonitrile. To avoid obstruction of the UPLC, microfiltration of standards and extracts was performed prior to injection through a 0.22 μm PVDF syringe filter (Membrane Solutions, Texas, USA) before being injected into the UPLC–MS system.

2.7. Validation study

For the validation study, the following parameters were evaluated in order to ensure the method quality: linearity, recovery, precision, limit of detection (LOD) and limit of quantification (LOQ). Linearity of the method was estimated by analysis of eight standard calibration solutions, injected in triplicate, in the range 1–50 μg/mL for all the sugars. Recoveries were calculated from the slope of the linear regression obtained between the added analyte concentration and the measured analyte concentration. The intraday (five replicates in same day) and inter-day (fifteen replicates for three consecutive days, each day 5 replicates) precisions were performed to evaluate the repeatability and reproducibility of the proposed method (Wabaidur, Alothman, & Khan, 2013). Limit of detection and limit of quantification were determined by spiked samples based on signal to noise ratio of 3:1 and 10:1, respectively.

3. Results and discussion

3.1. Optimization of chromatographic separation conditions

The development of a high throughput analytical technique for the analysis of saccharides in dates extract using UPLC–MS is of high interest. One of the major benefits of the UPLC columns, where particle size is <2.0 μm, is that the efficiency does not fall when increasing the flow rates. A flow rate of 0.4 mL/min was found to be optimal for the separation of saccharides, conditions that made possible to achieve an analysis time less than 7 min. Fig. 1 shows the chromatograms of the five standard saccharides obtained with the optimal working conditions. Different types of mobile phase, corresponding to various methanol/water and acetonitrile/water mixtures with different compositions, containing 0.1% formic acid or 0.1% ammonia as additive, were compared. The signal-to-noise ratio was improved using acetonitrile/water containing 0.1% ammonia (v/v) as mobile phase and this eluent was hence selected as mobile phase. The chromatographic separation of saccharides was also evaluated using different columns (C_8, C_{18} and amide). The best separation with good symmetry and resolution has been achieved with a BEH amide column (Fig. 1). BEH amide stationary phase has proven to be particularly useful for a rapid and efficient separation of underivatized sugars, because the retention of these carbohydrates is based on the hydrogen bonding interactions of the sugars hydroxyl groups with the amide groups of the stationary phase. Thus, the retention time predictably increases with the elongation of oligosaccharide chain. This system is characterized by its selectivity.

3.2. Optimization of ESI-MS conditions

The ESI-MS conditions were optimized by infusing standards of 5 μg/mL of each saccharide in either positive or negative ionization mode to desolvate efficiently the aqueous/organic mobile phase and provide the maximum analyte response. In positive mode, no signal was recorded under different ion source parameters. Then, the optimization was carried out in negative ionization mode, and highly abundant analytes signals were detected, thus the negative ionization mode was selected for mass spectrometric detection. The obtained narrow chromatographic peaks (5 s width) required a fast scanning analyzer to define the peaks with enough points such as the triple quadrupole used in this work. The effect of various ESI–MS parameters such as capillary voltage (2.5–4.5 kV), cone voltage (5–90 V), source temperature (100–150 °C), desolvation temperature (200–450 °C) and desolvation gas flow rate (400–800 L/h) was studied. The maximum analyte response was obtained using the following conditions: 3.0 kV of capillary voltage, 120 °C of source temperature, 300 °C of desolvation temperature and 600 L/h of desolvation gas flow rate. The optimization experiment has pointed out that the effects of the ESI–MS parameters exceeding the optimized values, were insignificant. Selected Ion Reaction (SIR) mode using Intellistart software was used in order to select the best cone voltage and most abundant m/z values for each analyzed component. The most abundant precursors and SIR parameters for each analyte are shown in Table 2.

3.3. Linearity of the proposed method

Using the developed method, the calibration curves were generated for all sugars. Calibration standard solutions of 1, 2, 3, 4, 5, 10, 20 and 50 μg/mL of each sugar were prepared in acetonitrile/ultrapure water (Milli-Q) solution (50:50). Under the optimal experimental conditions a linear relationship between peak area and the corresponding sugar concentration was established. The regression equation for all five analyzed compounds has shown correlation coefficient (r^2) values higher than 0.998. The response was found linear in the range of 1–50 μg/mL. The limit of detection and limit of quantification values for all the analyzed compounds were in the range of 0.25–0.69 μg/mL and 0.82–3.58 μg/mL, respectively. The relative standard deviation (RSD) was found to be less than 2.35% for all five analyzed compounds from 10 independent measurements. All data are summarized in Table 3.

3.4. Repeatability and reproducibility of the method

Repeatability and reproducibility of the method were studied to evaluate the performance of the proposed method. For repeatability (run-to-run precision), five replicates were carried out on standard solution of all sugars on the same day at the concentration level 10 μg/mL. To assess the reproducibility (day-to-day precision), 15 replicates were performed with the same solutions over three consecutive days (five replicates each day) (Wabaidur et al., 2013). High repeatability and reproducibility were achieved with relative standard deviations lower than 1.5% in all the five cases. These results confirmed that the proposed analytical method was successful in providing acceptable values of repeatability and reproducibility required for an accurate analysis of sugars. From these results, it could be concluded that the proposed UPLC–MS method can be successfully used in the routine analysis of sugars in various date samples.

3.5. Quantification of sugars in different varieties of date samples

The optimized UPLC–MS method was applied to quantify the amount of fructose, glucose, sucrose, kestose and nystose present
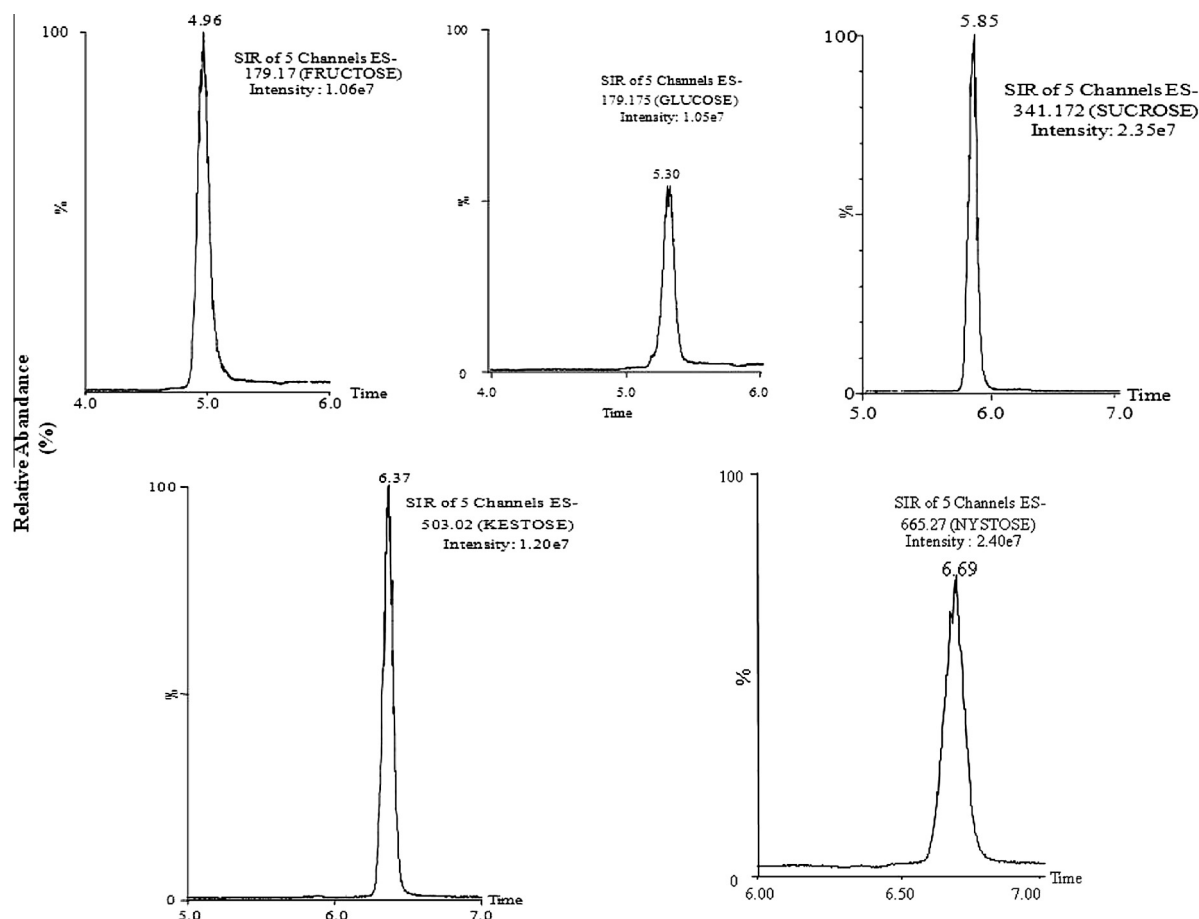
Fig. 1. The chromatographic separation peaks of saccharides in standard mixture – SIR record is displayed from calibration level 50 µg/mL, each chromatogram displays a specific SIR signal for an individual compound.

Table 2
Characteristics of target compounds and optimized Selected Ion Reaction (SIR) monitoring parameters for the studied saccharides.

Sugars	Molecular formula	MS parameters		
		Precursor ion $(M-H)^-$	Cone voltage (V)	Dwell time (s)
Fructose	$C_6H_{12}O_6$	179.170	18	0.025
Glucose	$C_6H_{12}O_6$	179.175	18	0.025
Sucrose	$C_{12}H_{22}O_{11}$	341.172	34	0.025
Kestose	$C_{18}H_{32}O_{16}$	503.020	44	0.025
Nystose	$C_{24}H_{42}O_{21}$	665.272	56	0.025

Table 3
The calibration parameters for the proposed method.

Compound	Linear regression equation (µg/mL)	Correlation coefficient (r^2)	LOD (µg/mL)	LOQ (µg/mL)	[a]RSD (%)
Fructose	$y = 12839x - 77,292$	0.9987	0.45	0.99	1.12
Glucose	$y = 47198x - 13,496$	0.9989	0.46	1.08	0.88
Sucrose	$y = 44760x + 16,351$	0.999	0.25	1.06	1.22
Kestose	$y = 31092x - 3978.7$	0.9986	0.69	2.38	1.32
Nystose	$y = 28737x - 4166.6$	0.9989	0.38	1.29	0.73

[a] RSD for 10 repeated measurements.

in three varieties of dates, Sefri, Mabroom and Ghassab which were obtained from local markets in Saudi Arabia. Fig. 2 shows the UPLC–MS chromatogram of Sefri date extract. The results for the concentration of sugars in the analyzed date samples are shown

in Table 4. The recovery test was carried out by standard addition method. Five replicates samples at low, medium and high quality control were prepared for recovery determination. The liquid chromatography–mass spectrometric analysis results of experimental samples showed that the fructose concentration was higher in Ghassab dates while glucose contents were higher in Sefri dates (Table 4). In Mabroom date species concentrations of monosaccharides and oligosaccharides were notably lower than in the two other date species. On the other hand sucrose and kestose amount were also higher in Ghassab date species.

The sugar composition of the three dates harvested in Saudi Arabia were compared to those obtained for five ripe dates cultivated in Tunisia in which the fructose and glucose contents were lower, in the ranges 14.01–27.80 and 14.72–29.77 g/100 g, respectively (El Arem et al., 2011). On the other hand, the sucrose level in our three Saudi dates was in the range 16.17–18.47 g/100 g, while it was not detected in two Tunisian species and showed high values in three varieties from 17.86 to 33.32 g/100 g, the latest concentration corresponding to Deglet Nour which is a famous variety reputed for its high sweetness. Comparison of the found values to those previously published for the "Boufeggous" dates cultivated in Morocco showed that in this species fructose and glucose were in the ranges 34–37 and 39–42 g/100 g, respectively, and thus clearly less than in the three studied Saudi species (Azelmat, ElGarrouj, Mouhib, & Sayah, 2006).

Cáceres, Cárdenas, Gallego, and Valcárcel (2000) have reported a spectrophotometric method for the determination of sugars in food samples. They analyzed the monosaccharides and oligosaccharides in various fruit juices and jams. According to their data
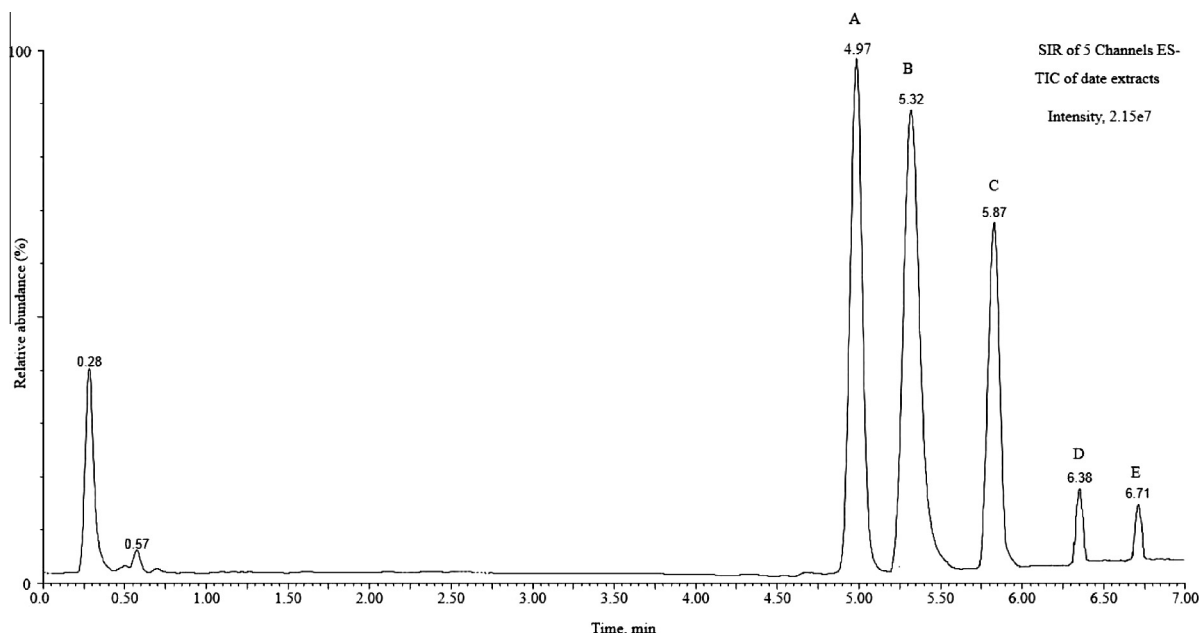
Fig. 2. UPLC–MS chromatograms of Sefri date extracts.

Table 4
Sugars level in the analyzed date Fruit samples ($n = 3$) (g/100 g dry wt.) and their estimated recovery rates.

Sugars	Sefri g/100 g ± SD[a]	Recovery (%)	Ghassab g/100 g ± SD[a]	Recovery (%)	Mabroom g/100 g ± SD[a]	Recovery (%)
Fructose	31.52 ± 2.5	90	33.56 ± 2.6	91	25.18 ± 2.1	92
Glucose	29.43 ± 2.3	90	28.10 ± 2.3	91	26.60 ± 2.2	93
Sucrose	16.17 ± 1.6	89	18.47 ± 1.9	90	16.25 ± 1.6	93
Kestose	0.96 ± 0.6	90	1.28 ± 1.1	90.	1.09 ± 1.0	93
Nystose	0.46 ± 0.3	89	0.37 ± 0.3	90	0.29 ± 0.4	92

SD = standard deviation obtained from addition standard calibration curve.
[a] Mean of three measurements.

it is clear that fruits contain not only monosaccharides, but also oligosaccharides. We have also compared the obtained results to the contents of several sugars in various fruits; the concentrations of fructose, glucose and sucrose in Saudi dates were always higher than those determined by HPLC in a variety of fruits, except for white and yellow peaches which showed a higher level of sucrose (Ma, Sun, Chen, Zhang, & Zhu, 2014; Shanmugavelan et al., 2013).

4. Conclusion

An efficient and highly sensitive UPLC–MS method has been developed for the simultaneous determination of fructose, glucose, sucrose, kestose and nystose. This work has focused on these compounds due to their importance. UPLC coupled with MS has shown to be an efficient alternative to traditional analytical techniques for the separation and characterization of underivatized sugars. The good performance of this method comes from different relevant aspects such as UPLC allows rapid analysis (with a chromatographic run time less than 7 min per sample), good linearity over a wide concentrations range, good reproducibility and improved sensitivity due to the narrower chromatographic peaks. The mass spectrometry allowed the acquisition of SIR monitoring of the compounds with good sensitivity by providing a reliable confirmation of sugars detected in samples. The developed method was successfully applied to the simultaneous determination of fructose,

glucose, sucrose, kestose and nystose in extracted samples of Sefri, Mabroom and Ghassab dates. The obtained results of the sugars suggest that the method proved to be an excellent tool for sensitive and quick analysis of sugars from biological matrices like dates extract with no need of derivatization.

References

Al-Abdoulhadi, I. A., Al-Ali, S., Khurshid, K., Al-Shryda, F., Al-Jabr, A. M., & Ben Abdallah, A. (2011). Assessing fruit characteristics to standardize quality norms in date cultivars of Saudi Arabia. *Indian Journal of Science and Technology, 4*, 1262–1266.

Ali, A., Al-Kindi, Y. S. M., & Al-Said, F. (2009). Chemical composition and glycemic index of three varieties of Omani dates. *International Journal of Food Sciences and Nutrition, 60*, 51–62.

Alpert, A. J., Shukla, M., Shukla, A. K., Zieske, L. R., Yuen, S. W., Ferguson, M. A. J., et al. (1994). Hydrophilic-interaction chromatography of complex carbohydrates. *Journal of Chromatography A, 676*, 191–202.

Azelmat, K., ElGarrouj, D., Mouhib, M., & Sayah, F. (2006). Irradiation of 'Boufeggous' dates: Effects on chemical composition during storage. *Postharvest Biology and Technology, 39*, 217–222.

Bekers, M. M., Marauska, M., Grube, M., Karklina, D., & Duma, M. (2004). New prebiotics for functional food. *Acta Alimentaria, 33*, 31–37.

Blanken, W. M., Bergh, M. L. E., Koppen, P. L., & Van den Eijnden, D. H. (1985). High-pressure liquid chromatography of neutral oligosaccharides: Effects of structural parameters. *Analytical Biochemistry, 145*, 322–330.

Brons, C., & Olieman, C. (1983). Study of the high-performance liquid chromatographic separation of reducing sugars, applied to the determination of lactose in milk. *Journal of Chromatography A, 259*, 79–86.

Cáceres, A., Cárdenas, S., Gallego, M., & Valcárcel, M. (2000). A continuous spectrophotometric system for the discrimination/determination of monosaccharides and oligosaccharides in foods. *Analytica Chimica Acta, 404*, 121–129.

Cheng, Y. L., & Lee, T. Y. (1992). Separation of fructose and glucose mixture by zeolite Y. *Biotechnology and Bioengineering, 40*, 498–504.

Churms, S. C. (1996). Recent progress in carbohydrate separation by high-performance liquid chromatography based on size exclusion. *Journal of Chromatography A, 720*, 151–166.

El Arem, A., Flamini, G., Saafi, E. B., Issaoui, M., Zayene, N., Ferchichi, A., et al. (2011). Chemical and aroma volatile compositions of date palm (*Phoenix dactylifera* L.) fruits at three maturation stages. *Food Chemistry, 127*, 1744–1754.

Goulas, A. K., Kapasakalidis, P. G., Sinclair, H. R., Rastass, R. A., & Grandison, A. S. (2002). Purification of oligosaccharides by nanofiltration. *Journal of Membrane Science, 209*, 321–335.

Huisman, M. M. H., Brull, L. P., Thomas-Oates, J. E., Haverkamp, J., Schols, H. A., & Voragen, A. G. (2001). The occurrence of internal (1, 5)-linked arabinofuranose and arabinopyranose residues in arabinogalactan side chains from soybean pectic substances. *Journal of Carbohydrate Research, 330*, 103–114.

Kafkas, E., Koşar, M., Tüemiş, N., & Başer, K. H. C. (2006). Analysis of sugars, organic acids and vitamin C contents of blackberry genotypes from Turkey. *Food Chemistry, 97*, 732–736.

Klein, A., Carnoy, C., Lo-Guidice, J. M., Lamblin, G., & Roussel, P. (1992). Separation of mucin oligosaccharide-alditols by high performance liquid chromatography on alkylamine-bonded silica columns. Effects of structural parameters. *Carbohydrate Research, 236*, 9–16.

Li, W. Y., Li, J., Shen, J., & Chen, C. X. (2002). Study on purifying fructooligosaccharides by membrane separation, in: *Proceedings of the 4th National Membrane Science and Technology Conference Report*, Nanjing, PR China, pp. 10.

Lin, S. C., & Lee, W. C. (1998). Separation of a fructo-oligosaccharide mixture by hydrophilic interaction chromatography using silica-based micropellicular sorbents. *Journal of Chromatography A, 803*, 302–306.

Liu, Y., Urgaonkar, S., Verkade, J. G., & Daniel, W. (2005). Separation and characterization of underivatized oligosaccharides using liquid chromatography and liquid chromatography–electrospray ionization mass spectrometry. *Journal of Chromatography A, 1079*, 146–152.

Ma, C., Sun, Z., Chen, C., Zhang, L., & Zhu, S. (2014). Simultaneous separation and determination of fructose, sorbitol, glucose and sucrose in fruits by HPLC-ELSD. *Food Chemistry, 145*, 784–788.

Mabel, M. J., Sangeetha, P. T., Platel, K., Srinivasan, K., & Prapulla, S. G. (2008). Physicochemical characterization of fructooligosaccharides and evaluation of their suitability as a potential sweetener for diabetics. *Carbohydrate Research, 343*, 56–66.

Macrae, R. (1988). *HPLC in Food Analysis*. London: Academic Press Limited (pp. 86).

Myhara, R. M., Karkalas, J., & Taylor, M. S. (1999). The composition of maturing Omani dates. *Journal of the Science of Food and Agriculture, 79*, 1345–1350.

Nikolov, Z. L., & Reilly, P. J. (1985). Retention of carbohydrates on silica and amine-bonded silica stationary phases: Application of the hydration model. *Journal of Chromatography A, 325*, 287–293.

Ohta, A., Ohtsuki, M., Baba, S., Takizawa, T., Adachi, T., & Kimura, S. (1995). Effects of fructooligosaccharides on the absorption of iron, calcium and magnesium in iron-deficient anemic rats. *Journal of Nutritional Science and Vitaminology, 42*, 281–291.

Peris, M. (1996). In L. M. L. Nollet (Ed.), *Handbook of food analysis* (pp. 533–550). New York: Marcel Dekker.

Rabel, F. M., Caputo, A. G., & Butts, E. T. (1976). Separation of carbohydrates on a new polar bonded phase material. *Journal of Chromatography A, 126*, 731–740.

Raschka, L., & Daniel, H. (2005). Mechanisms underlying the effects of inulin-type fructans on calcium absorption in the large intestine of rats. *Bone, 37*, 728–735.

Sahari, M. A., Barzegar, M., & Redfar, R. (2007). Effect of varieties on the composition of dates (*Phoenix dactylifera* L.) – Note. *Food Science and Technology International, 13*, 269–275.

Sakai, K., Ohta, A., Takasaki, M., & Tokunaga, T. (2000). The effect of short chain fructooligosaccharides in promoting recovery from post-gastrectomy anemia is stronger than that of inulin. *Nutrition Research, 20*, 403–412.

Sakr, M. M., Abu Zeid, I. M., Hassan, A. E., Baz, A. G. I. O., & Hassan, W. M. (2010). Identification of some date palm (*Phoenix dactylifera*) cultivars by fruit characters. *Indian Journal of Science and Technology, 3*, 338–343.

Shanmugavelan, P., Kim, S. Y., Kim, J. B., Kim, H. W., Cho, S. M., Kim, S. N., et al. (2013). Evaluation of sugar content and composition in commonly consumed Korean vegetables, fruits, cereals, seed plants, and leaves by HPLC-ELSD. *Carbohydrate Research, 380*, 112–117.

Stefansson, M., & Westerlund, D. (1996). Ligand-exchange *chromatography* of carbohydrates and glycoconjugates. *Journal of Chromatography A, 720*, 127–136.

Vågen, I. M., & Slimestad, R. (2008). Amount of characteristic compounds in 15 cultivars of onion (*Allium cepa* L.) in controlled field trials. *Journal of the Science of Food and Agriculture, 88*, 404–411.

Varki, A. (1993). Biological roles of oligosaccharides: All of the theories are correct. *Glycobiology, 3*, 97–130.

Wabaidur, S. M., Alothman, Z. A., & Khan, M. R. (2013). A rapid method for the simultaneous determination of L-ascorbic acid and acetylsalicylic acid in aspirin C effervescent tablets by ultra performance liquid chromatography–tandem mass spectrometry. *Spectrochimica Acta A: Molecular and Biomolecular Spectroscopy, 108*, 20–25.

Wang, X. L., Zhang, C. H., & Ouyang, P. K. (2002). The possibility of separating saccharides from a NaCl solution by using nanofiltration in diafiltration mode. *Journal of Membrane Science, 204*, 271–281.

Westman, E. C. (2002). Is dietary carbohydrate essential for human nutrition? *The American Journal of Clinical Nutrition, 75*, 951–953.