Research Article

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Determination of the variability of sugars in date fruit varieties

Chuan-Rui Zhang[1], Saleh A. Aldosari[2]*, Polana S.P.V. Vidyasagar[2], Paraj Shukla[2] and Muraleedharan G. Nair[1, 2]

[1]*Bioactive Natural Products and Phytoceuticals Laboratory, Department of Horticulture, Michigan State University, East Lansing MI, 48824, USA*

[2]*Chair of Date Palm Research, Department of Plant Protection, College of Food and Agriculture Sciences, King Saud University, Riyadh 11451, Saudi Arabia*

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Abstract

The consumption of date fruit has increased around the world. To meet the demand, numerous varieties of date palms are under commercial production. In this study, total sugars in 29 varieties of date fruits produced in Saudi Arabia were quantified and characterized. The quantification of sugars was done by HPLC using a carbohydrate column and RI detector. Structures of sugars were characterized by NMR methods. Total sugar content in 29 varieties of the dried date fruits ranged from 61.7 to 78.6 per cent. Among these, only Deglet Noor, Sukkari Al Qassim and Nabtat Ali dates contained sucrose. The rest of the varieties showed higher levels of fructose than glucose and were devoid of sucrose. The fructose and glucose existed as a mixture of β-D-fructopyranose and β-D-fructofuranose; and α-D-glucopyranose and β-D-glucopyranose, respectively. Date fruits contain only fructose, glucose and sucrose as carbohydrates. All date fruits in this study, except Sukkari Al Qassim and Deglet Noor, showed higher levels of fructose than other sugars, which support the health benefits of date fruits as dietary components.

Keywords: Date palm, fructose, glucose, *Phoenix dactylifera*, sucrose

Introduction

Date palm, *Phoenix dactylifera* L. (Palmaceae), cultivation is primarily in the Middle East, North Africa, South Asia and USA. Date fruit is one of the dietary ingredients for many in several countries. Its health benefits are accounted primarily due to its rich sugar content, as well as dietary fiber, amino acids, vitamins, and minerals (Fayadh and Al-Showiman, 1990; Hamada *et al*., 2002).

The current study is focused on the date fruits produced in Saudi Arabia. Over 450 date palm varieties or cultivars are grown in the Kingdom of Saudi Arabia with an annual yield of over 1 million metric tonnes of date fruits. About 25 million date palm trees are grown in Saudi Arabia covering an estimated 1,57,000 ha area, about 14 per cent of the total world production (FAOSTAT, 2014). In this study, we selected 29 significant commercial varieties of date fruits collected from farms in Saudi Arabia. We had earlier reported the bioactive components in Ajwa date fruit (Zhang *et al*., 2013). This prompted us to investigate the varietal difference in the sugar content of significant commercial varieties of date fruits such as Barni Al Madinah, Hulwa, Khashram, Khodry, Khalas, Deglet Noor, Dekhaini, Rabeaa, Rushodia, Ruthana, Ruthana Al Sharag, Sabaka, Sukkari Al Qassim, Sullaj, Shalabi, Shaishee, Safawi, Sefri, Segae, Ajwa, Anbara, Luban, Mabroom, Majhool, Mutwah, Meneifi, Nabtat Ali and Naboot Seif, Hilali (Table 1).

**Corresponding Author: aldosari@ksu.edu.sa*

Materials and methods

Quantification of sugars was carried out on Waters 2010 HPLC system (Waters Corp., Milford, MA, USA) equipped with Empower Software, Shodex Degasser, Auto sampler (Waters 717), refractive index detector (RID) (Waters 2410, Waters Corporation, Milford, MA) and Waters high performance carbohydrate column (4.6x250 mm, 4 µm). Silica gel plates were used for TLC (200 µm) and preparative TLC (250 µm) (Analtech Inc., Newark, DE). All solvents used were of high-performance liquid chromatography (HPLC) grade (Sigma-Aldrich Chemical Company, St. Louis, MO, USA).

Table 1. Particulars on date palm varieties and its fruits

Date variety	Plant ID	Main regions of cultivation	Size/shape	Color
Barni Al Madinah	26	Madinah	Medium/long cylindrical	Brown
Hulwa	64	Al Jouf, Hail, Madinah, Qassim, Tabouk, Riyadh	Medium/long, cylindrical	Dark
Khashram	80	Riyadh	Medium/cylindrical	Dark red
Khodry	86	Aseer, Jazan, Madinah, Makkah, Qassim, Riyadh	Large/elliptical	Dark brown
Khalas	89	Eastern Region, Northern Borders, Qassim, Riyadh	Medium/long, cylindrical	Brown
Deglet Noor	98	Madinah, Riyadh	Medium/long, elliptical	Light brown
Dekhaini	100	Riyadh	Medium/cylindrical	Dark brown
Rabeaa	111	Madinah, Makkah	Large/oval	Dark brown
Rushodia	114	Qassim, Riyadh	Medium to large/long, cylindrical	Light brown
Ruthana	116	Aseer, Madinah, Qassim, Riyadh	Medium/oval	Yellowish brown
Ruthana Al Sharag	117	Madinah	Medium/oval	Yellowish brown
Sabaka	124	Qassim, Riyadh	Medium/long, cylindrical	Light brown
Sukkari Al Qassim	138	Qassim	Medium/long, cylindrical	Brown
Sullaj	142	Aseer, Northern Borders, Riyadh	Medium/long, elliptical	Maroon
Shalabi	159	Madinah	Large/cylindrical	Dark brown
Shaishee	164	Eastern Region	Medium to large/oval	Reddish brown
Safawi	169	Madinah	Medium to large/elliptical	Dark red
Sefri	173	Aseer, Riyadh	Medium/elliptical	Brown
Segae	176	Aseer, Northern Borders, Qassim, Riyadh	Medium to large/oval	Reddish brown
Ajwa	185	Madinah	Medium/oval	Dark
Anbara	191	Madinah	Large/long	Maroon red
Luban	226	Madinah	Medium/oval	Brown
Mabroom	231	Madinah	Medium to large/long	Brown
Majhool	234	Madinah	Small to large/long	Reddish brown
Mutwah	246	Riyadh	Medium/cylindrical	Dark red
Meneifi	254	Riyadh	Medium/oval	Light brown
Nabtat Ali	273	Qassim	Medium to large/oval	Maroon
Naboot Seif	275	Central Region	Medium to large/oval	Brown
Hilali	285	Eastern Region, Riyadh	Large/oval	Brown

Date fruit samples

Twenty nine varieties of date fruit samples were collected from the commercial date farms of Madinah, Qassim, Hail, Al Hassa, Riyadh and Al Kharj regions of Saudi Arabia. Amongst them, Barni Al Madinah, Hulwa, Dekhaini, Rabeaa, Rushodia, Ruthana, Ruthana Al Sharag, Sabaka, Sukkari Al Qassim, Shalabi, Shaishee, Safawi, Anbara, Luban, Mabroom, Mutwah and Hilali were collected on November 15, 2011; Khashram was collected on June 20, 2012; Khodry, Khalas, Deglet Noor, Sullaj, Sefri, Ajwa, Majhool, Meneifi, Nabtat Ali and Naboot Seif were collected on May 10, 2014; Segae was collected on April 26, 2014. The identity of each variety of fruit was established based on the information provided by the farmer and as per the database kept in the Ministry of Agriculture, Saudi Arabia (Table 1) (Anonymous, 2006). For each cultivar, 2 kg fruits were randomly collected in triplicate from thirty 2 kg packs of freshly picked date fruits at each farm. The 6 kg fruits so collected were then thoroughly mixed and a 1 kg sample was randomly prepared from the bulk for shipping to Michigan State University for analyses. The farms visited for collection of fruits had minimum of 10,000 palm trees. Each date palm provides between 30 and 90 kg of fruit from 10 to 12 bunches, depending on the cultivar. The shipment of fruits upon arrival at Michigan State University was kept at -20 °C till analyses.

To quantify sugar content in date fruit varieties, all 29 varieties of date fruits were weighed separately and pitted. The pit-free date fruits were cut in small pieces, homogenized with water (100 mL), allowed to stand (1h) at room temperature and then centrifuged (10,000 rpm, 15 min). An aliquot (500 µL) of the resulting supernatant was diluted with water (500 µL) and then with acetonitrile (3 mL) to afford a total volume of 4 mL. An aliquot (1 mL) of this solution was filtered through a 0.2 µm PTFE membrane prior to analysis by HPLC.

Quantification of sugars in date fruits by HPLC

The sugars in each date fruit were separately identified and quantified by HPLC using a carbohydrate column under refractive index (RI) detection. The carbohydrate column used for the analyses was maintained at 35 °C using a column heater module (Waters Corporation, Milford, MA). The C_{18} guard column cartridge (Waters Corporation, Milford, MA) was replaced after every 100 injections. An aliquot of 25 µL of standards and test solutions was injected and eluted with premixed solvent system (water-acetonitrile, 15:85 v/v) at a flow rate of 1.75 mL min^{-1} under isocratic condition. In between injections, the column was equilibrated for 3 min using the same solvent system. The attenuation of RI detector was maintained at 8 throughout the analyses. The standard solutions of fructose, dextrose (glucose monohydrate) and sucrose (Sigma-Aldrich, St. Louis, MO) were prepared by using 20, 15, 10, 5 and 2.5 mg mL^{-1} concentrations respectively, and analyzed in duplicate. The analyses were carried out in three different times for all samples. Calibration curves (Fig. 1) were obtained by plotting the average of the mean peak areas of duplicate runs from three independent experiments against concentrations. The calibration plot for glucose was calculated based on the glucose content in dextrose. Each extract was analyzed in duplicate. The mean peak areas from the duplicate analyses were used to read the concentration of fructose, glucose and sucrose from their respective calibration curves. For each date variety, three such independent experiments were carried out. The data collected were then averaged to determine the quantity of fructose, glucose and sucrose in each variety. It is important to note that quantification was limited to fructose, glucose and sucrose since all date fruits studied showed the presence of only these three sugars based on initial HPLC profiles.

NMR spectrum of sugars

i) Fructose

White powder; ^{1}H NMR (500 MHz, D_2O): δ 4.16 (m, fur H-3, 4), 4.09 (m, pyr H-5, 6a), 3.95 (m, pyr H-4), 3.88 (m, fur H-5, 6a), 3.84 (m, pyr H-3), 3.76 (m, pyr H-1a, 6b), 3.72 (m, fur H-6b), 3.64 (m, fur H-1a), 3.61 (m, pyr H-1b), 3.60 (m, fur H-1b). According to the spectral data, the fructose was identified as the mixture of β-D-fructopyranose and β-D-fructofuranose (Barclay *et al*., 2012) (Fig. 2).

ii) Glucose

White powder; ^{1}H NMR (500 MHz, D_2O): δ 5.28 (m, α H-1), 4.69 (m, β H-1), 3.86-3.94 (m, β H-4, 6a, α H-4, 6a), 3.74-3.84 (m, β H-6b,

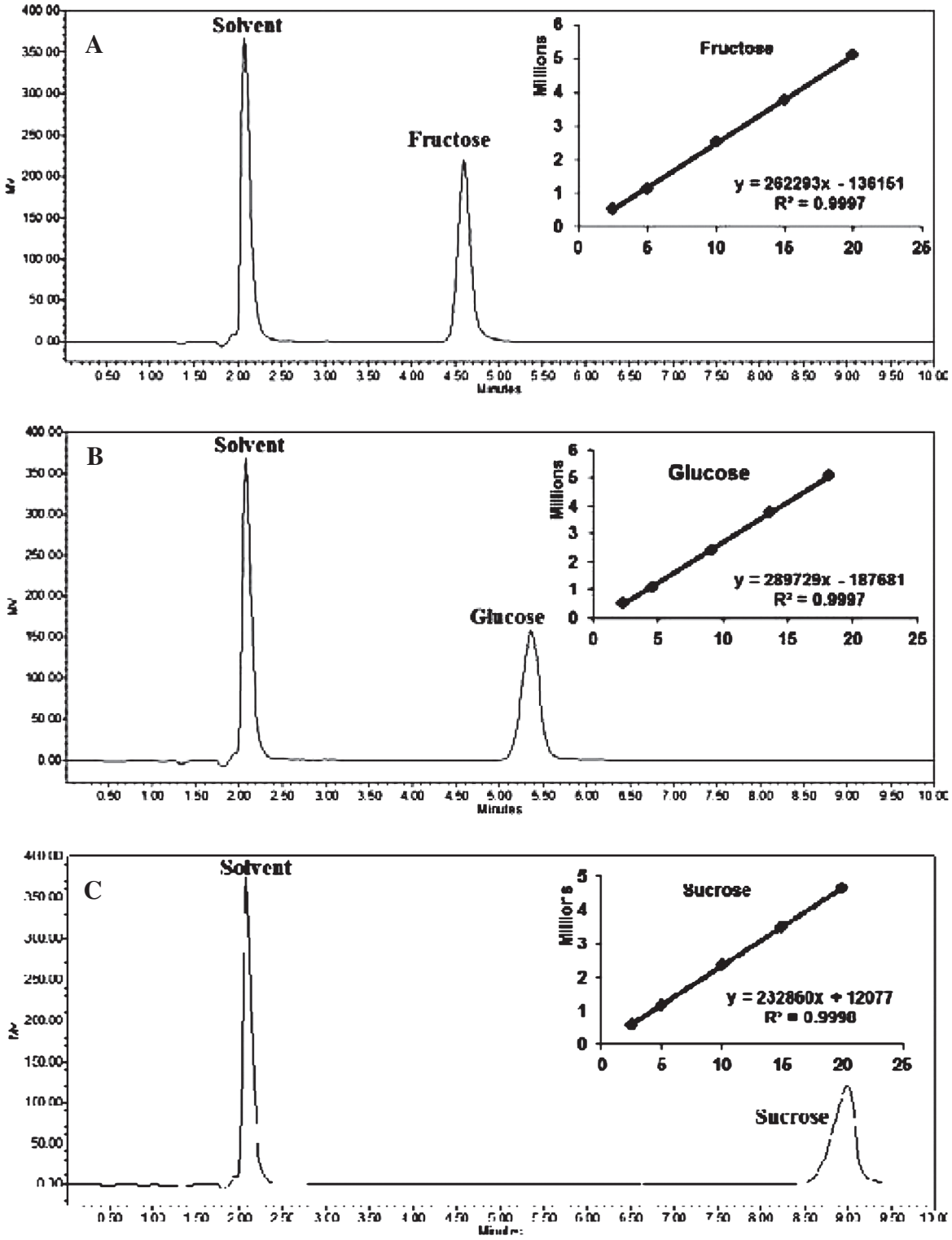

Fig. 1. HPLC profiles and calibration curves obtained by plotting the average of the mean peak areas from duplicates of three independent experiments against concentrations, (A) fructose (rt. 4.59 min), (B) glucose (rt. 5.29 min) and (C) sucrose (rt. 8.91 min).

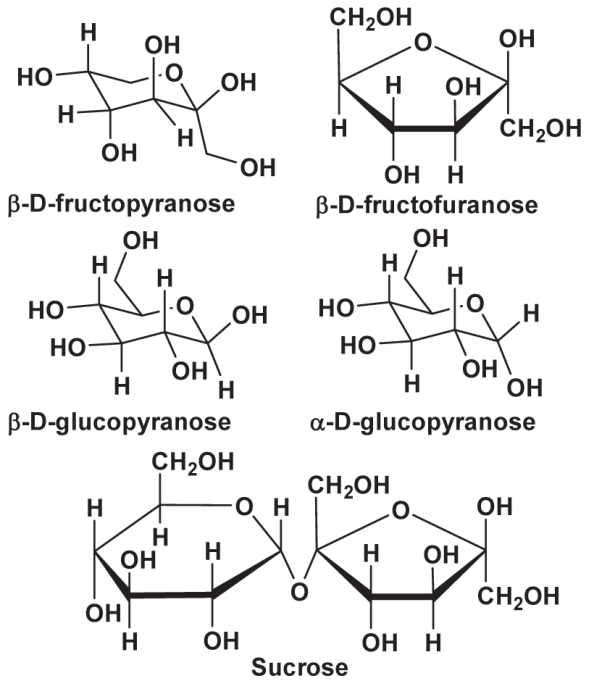

Fig. 2. Structures of fructose, glucose and sucrose as confirmed by NMR experiments

α H-3, 6b), 3.42-3.60 (m, β H-3, 5, α H-2, 5), 3.29 (m, β H-2). According to the spectral data, the glucose was identified as the mixture of β-D-glucopyranose and α-D-glucopyranose (Gurst, 1991) (Fig. 2).

iii) Sucrose

White powder; ^{1}H NMR (500 MHz, D_2O): δ 5.46 (d, *J* = 3.7 Hz, H-1), 4.26 (d, *J* = 8.8 Hz, H-3′), 4.10 (t, *J* = 8.6 Hz, H-4′), 3.79-3.94 (m, H-3, 4, 6a, 6b, 5′, 6′ a, 6′ b), 3.72 (s, H-1′ a, 1′ b), 3.62 (dd, *J* = 9.9, 3.7 Hz, H-2), 3.52 (t, *J* = 9.4 Hz, H-5); ^{13}C NMR: δ103.7 (C-2′), 92.2 (C-1), 81.4 (C-5′), 76.5 (C-3′), 74.0 (C-4′), 72.6 (C-3), 72.4 (C-5), 71.1 (C-2), 69.2 (C-4), 62.4 (6′), 61.4 (C-1′), 60.1 (C-6). According to the spectral data, the sucrose was identified as the α-D-glucopyranose, (1→2) β-D-fructofuranose (De Bruyn and Van Loo, 1991) (Fig. 2).

Results and discussion

Ajwa and Anbara are two of the best cultivars of Madinah region. The latter is cultivated on a limited scale. The cultivar Shalabi is a preferred variety for drying and packing. From Qassim region, Sukkari Al Qassim and Nabtat Ali are two important cultivars while Khalas is the best commercial variety from Eastern region. From Riyadh region, Segae has a major share in terms of production, processing and consumption.

In general, the fruits of the cultivars Khalas, Khodry, Majhool, Nabtat Ali and Shalabi have a good texture and less fiber content which are important attributes for high palatability. The other seven fruits in high demand are Ajwa, Mabroom, Rabeaa, Safawi, Shaishee and Sullaj. However, the cultivars which occupy major share in the commerce are Majhool, Ajwa, Mabroom, Segae, Deglet Noor, Khodry and Khalas. Out of the 29 cultivars included in this study, Anbara, Hilali, Khodry, Rabeaa and Shalabi bear large fruits followed by Deglet Noor, Mabroom, Naboot Seif, Nabtat Ali, Rushodia, Segae and Shaishee, the fruits of which vary from medium to large. The rest of the 17 cultivars yield medium size fruits except Majhool where fruits vary from small to large (Table 1).

Date fruits exhibit high degree of diversity in its color. For example, Ajwa and Hulwa were the darkest fruits included in this study. The cultivars Khodry, Rabeaa, Shalabi and Dekhaini were dark brown, whereas Khashram, Mutwah and Safawi dark red. The maroon colored fruits were from cultivars such as Sullaj and Nabtat Ali. In general, the predominant fruit color amongst all the cultivars was brown (Table 1). It is important to note that at the first ripening stage, 'Bisr' or 'Khalal', fruits in general, were light colored and as they ripen, they become darker. On the basis of fruit color at 'Bisr' stage, the 29 cultivars included in this study can be classified into mainly two colors: yellow and red. However, except nine cultivars bearing red or reddish fruits, all of them bear yellow or yellowish fruits at the 'Bisr' stage of ripening (Hong *et al*., 2006).

The five cultivars, Hilali, Rushodia, Sullaj, Ruthana Al Sharag and Ruthana, out of 29 included in this study are preferred for consumption at only 'Rutab' stage, which is the middle stage flanked by 'Bisr' as the first stage and 'Tamar' as the final ripening stage. Whereas, nine cultivars preferred for consumption at the final ('Tamar') stage of ripening include, Ajwa, Sefri, Segae, Majhool, Khodry, Anbara, Shalabi, Safawi and Luban. The fruits of rest of the cultivars are consumed at both 'Rutab' and 'Tamar' stages, except Hulwa and

Khalas, whose fruits are liked at all three stages of ripening. The early maturing cultivars included in this study were Majhool, Ruthana Al Sharag, Ruthana, Dekhaini, Khashram, Mutwah, Mabroom and Rabeaa, while in case of cultivar Sefri, the maturity period varies from early to mid-season. The cultivars Deglet Noor, Barni Al Madinah, Khodry, Hilali, Meneifi and Anbara are all late maturing cultivars. Rest of the cultivars matures during mid-season which is the month of August. On the basis of moisture content at the final ('Tamar') stage of ripening, the date fruits can be classified into dry, semi-dry and soft.

Table 2. Fructose, glucose and sucrose present in date fruits varieties studied (g 100 g^{-1} date)

Date fruit variety	Fructose	Glucose	Sucrose
Barni Al Madinah	38.69±0.16	34.82±0.19	nd
Hulwa	36.20±0.03	33.52±0.12	nd
Khashram	34.56±0.06	31.21±0.20	nd
Khodry	37.12±0.09	33.33±0.23	nd
Khalas	37.42±0.36	34.42±0.31	nd
Deglet Noor	22.00±0.05	21.07±0.13	31.17±0.16
Dekhaini	36.91±0.03	33.64±0.14	nd
Rabeaa	37.30±0.22	34.33±0.07	nd
Rushodia	32.38±0.16	29.32±0.14	nd
Ruthana	34.58±0.24	31.62±0.10	nd
Ruthana Al Sharag	39.80±0.27	36.27±0.13	nd
Sabaka	35.09±0.15	31.61±0.05	nd
Sukkari Al Qassim	10.09±0.07	10.07±0.28	43.51±0.34
Sullaj	39.19±0.20	35.95±0.09	nd
Shalabi	36.78±0.13	33.30±0.19	nd
Shaishee	37.50±0.25	34.60±0.20	nd
Safawi	40.80±0.27	37.15±0.19	nd
Sefri	36.47±0.08	34.05±0.08	nd
Segae	36.94±0.30	34.70±0.33	nd
Ajwa	33.35±0.12	29.50±0.12	nd
Anbara	36.87±0.08	33.04±0.12	nd
Luban	33.92±0.25	31.05±0.12	nd
Mabroom	41.12±0.16	37.50±0.09	nd
Majhool	33.90±0.44	31.43±0.32	nd
Mutwah	38.21±0.23	33.78±0.02	nd
Meneifi	34.42±0.14	31.55±0.28	nd
Nabtat Ali	23.58±0.12	21.53±0.06	16.82±0.10
Naboot Seif	38.50±0.26	34.13±0.27	nd
Hilali	38.32±0.08	34.91±0.10	nd

nd: not detected

Most date fruits are harvested and consumed at both rutab and tamar stages when they are brown or red in color with high sugar content. The predominant sugars in date fruit are glucose, fructose and sucrose, whereas, sucrose, also known as invert sugar, is partly or completely converted into glucose and fructose depending on the stage of the fruit development and the date palm variety (Vayalil, 2011; Rastegar *et al*., 2012; Al-Massallem *et al*., 2013). The date fruits selected in this study were in the tamar stage.

The HPLC quantification data of sugars revealed that the total sugar content in these varieties ranged from 61.7 to 78.6 per cent on dry weight basis of the fruit and excluding the pit (Table 2). Among them, 26 varieties showed only monosaccharides, fructose and glucose, between 32.4 and 41.1 per cent and 29.3 and 37.5 per cent, respectively (Fig. 3). However, Deglet Noor, Sukkari Al Qassim and Nabtat Ali contained sucrose, a disaccharide (31.2, 43.5 and 16.8 per cent, respectively, Fig. 4) in addition to fructose (22, 10.1 and 23.6%) and glucose (21.1, 10.1 and 21.5%), respectively. In general, sucrose is completely converted into glucose and fructose by the invertase enzyme at rutab and tamar stages for most varieties of date fruit (Vayalil, 2011; Rastegar *et al*., 2012; Al-Massallem *et al*., 2013). Mabroom and Rushodia are examples of date fruits with the highest and lowest sugar content, 78.6 and 61.7 per cent, respectively. Unlike Nabtat Ali, Deglet Noor and Sukkari Al Qassim showed lower content of fructose and glucose but higher level of sucrose. The retention times for fructose, glucose and sucrose were 4.59, 5.29 and 8.91 min, respectively. It is important to note that fructose level was higher than glucose among monosaccharides in all varieties of date fruits analyzed.

The water extract from the variety Sukkari Al Qassim was used to isolate the three sugars for NMR studies. Pure sugars were obtained by the preparative TLC (silica gel plates, 250 µm, $CHCl_3$:MeOH:H_2O, 2/1/0.1 v/v/v, single run). The identities of the isolated sugars were confirmed by ^{1}H- and ^{13}C-NMR experiments as fructose (mixture of β-D-fructopyranose and β-D-fructofuranose) (Barclay *et al*., 2012), glucose (mixture of

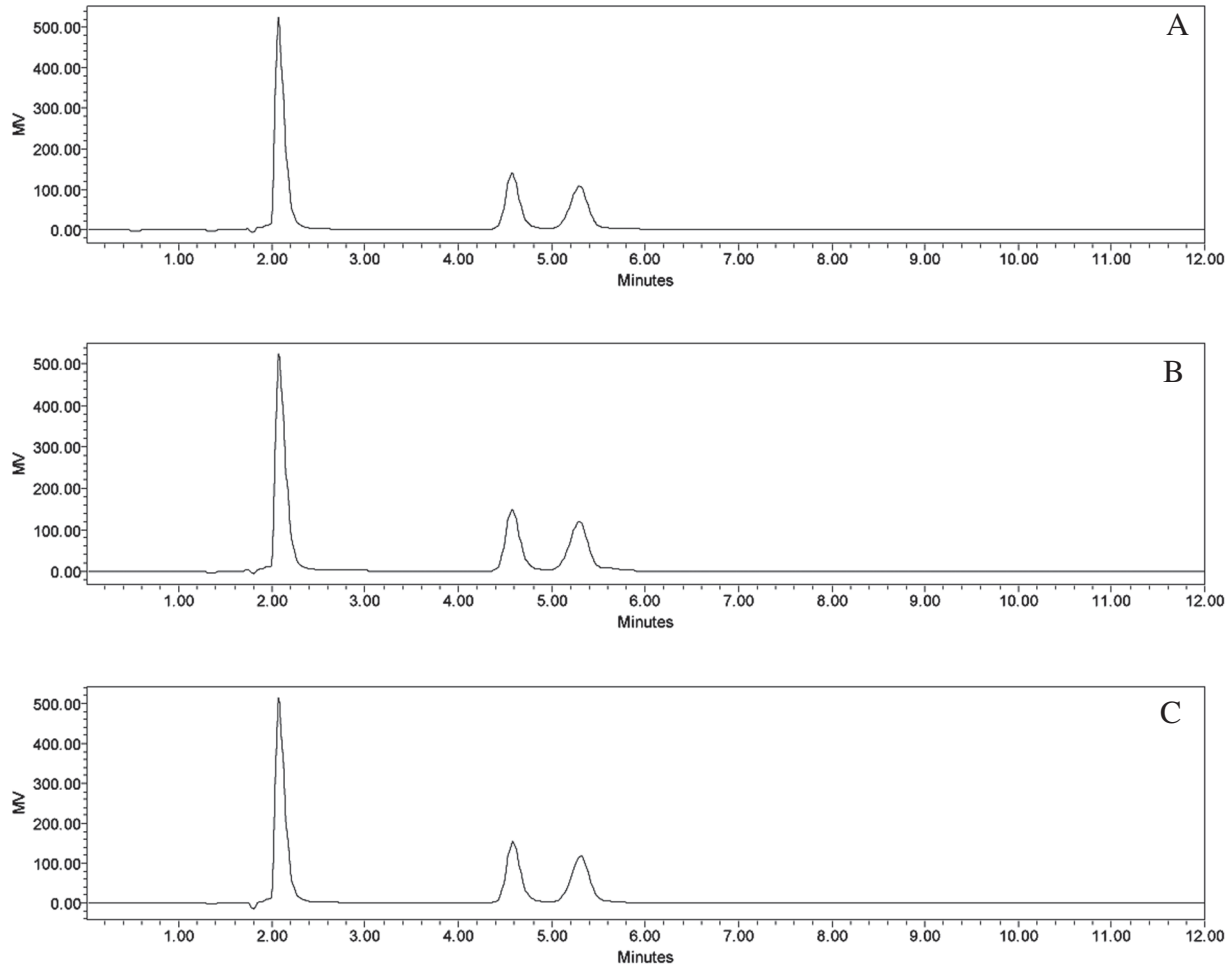

Fig. 3. HPLC profiles of sugars detected in date fruits (A) Ajwa (B) Khalas and (C) Segae. The other 23 varieties have similar profiles. All of these varieties did not contain sucrose.

α-D-glucopyranose and β-D-glucopyranose) (Gurst, 1991) and sucrose (α-D-glucopyranose, (1→2) β-D-fructofuranose) (De Bruyn and Van Loo, 1991). We did not isolate sugars from other varieties since all varieties showed HPLC profiles and retention times 4.59, 5.29 and 8.91 min, respectively, which matched with fructose, glucose and sucrose standards.

As most of the fruit is consumed locally, there are certain preferences for date varieties in each region of Saudi Arabia. For example in the Eastern region, the most popular variety is Khalas whereas, Sukkari is the most important date variety in the Central region. In Medina region the predominant cultivar is Ajwa which fetches a premium compared to others because of its religious significance. In general, most of the farmers grow diverse cultivars with respect to their color, texture, taste and yield. All date fruits in this study, except Sukkari Al Qassim and Deglet Noor, showed higher levels of fructose than other sugars. Deglet Noor, Sukkari Al Qassim and Nabtat Ali contained sucrose, which is most likely due to low invertase enzyme activity compared to other varieties. Notably, three of these sucrose-containing cultivars yield semi-dry fruits.

A recent genomic study of date palm varieties, *P. dactylifera*, suggested that genomic duplication is wide spread among date palm varieties (Al-Massallem *et al*., 2013). By characterizing the entire genome of Khalas variety, along with several other cultivars produced in the Al Qassim region of Saudi Arabia, it has been demonstrated that genes

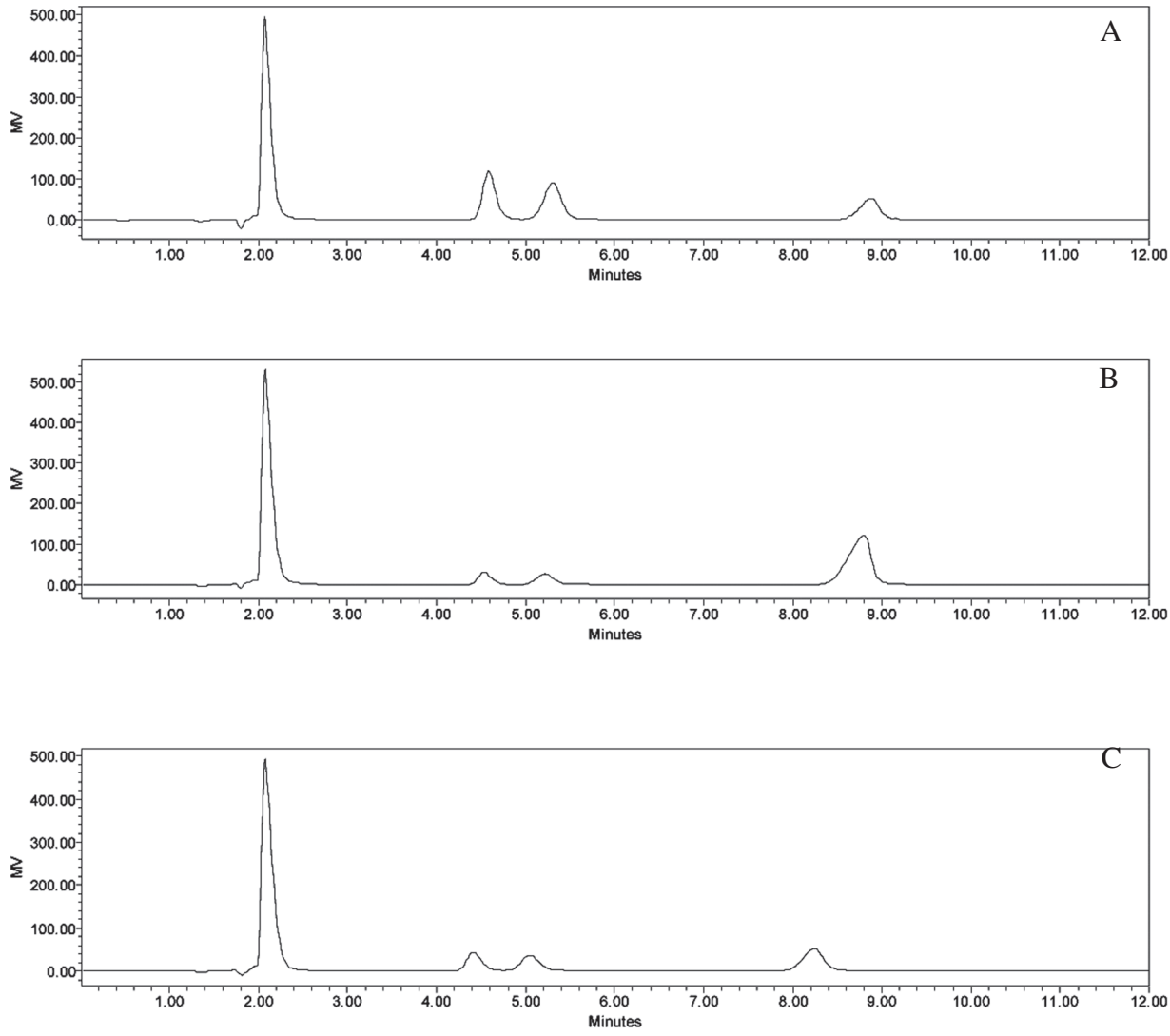

Fig. 4. HPLC profiles of sugars detected in date fruits (A) Nabtat Ali (B) Sukkari Al Qassim and (C) Deglet Noor. Only these varieties contained sucrose.

responsible for sugar metabolism are concentrated in the chromosomal region with low levels of single-nucleotide polymorphisms (Al-Massallem *et al*., 2013). The availability of invertase enzyme, the enzyme responsible for directing the equilibrium between sucrose and fructose/glucose at maturity of date fruit is critical for the fruit quality. The up- or down-regulation of invertase enzyme could be a function of the genetic variability of the date palm variety. In other words, the three date palm varieties with fruits showing sucrose content may have down-regulated the invertase enzyme during maturity of its fruit.

Conclusion

Our results suggest that date fruits in this study contained only fructose, glucose and sucrose as carbohydrates and were free from other pentoses, hexoses and oligosaccharides. This is the first report on the quantification and characterization of sugars in the varieties of date fruits produced in Saudi Arabia.

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