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RESEARCH PAPER

Evaluation of Several Chromatographic Resins on the Separation of Dates Sugar and their Impact on other Compounds in Dates

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تقييم فعالية عدد من الراتنجات الكروماتوجرافية في فصل سكر التمر و تأثير ذلك على المركبات الأخرى الموجودة في التمر

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Abstract. Dates are one of the most important food commodities in the Middle East countries, including the Sultanate of Oman. The fruit is regarded as a highly nutritious and healthy food. However, there are two million tons per year of dates that are abandoned waste worldwide. The objective was to establish an optimized method to separate sugars from dates using chromatographic resins and know the effect of those resins on the other ingredients. Fard date and three strong-cation gel-type resins (DOW-Ca, PCR-Ca and PCR-Na) were used in this study. The free-sugar extracts were analyzed by HPLC-MS method to quantify sugars and polyphenols. Most of the sugars were adsorbed by all of the tested resins with a higher selectivity towards fructose compared to glucose and sucrose. DOW-Ca had the lowest sugar adsorption compared to the others. Minerals profile by ICP detected a sharp reduction in potassium content, which was the main mineral found in *Fard* dates. Polyphenols content showed drastic decrease after treatment. Overall, the project succeeded in defining a method to remove sugar from dates and extend the loss of minerals and polyphenols in the process. For future work, it is recommended to evaluate the efficiency of other chromatographic resins in sugar separation.

Keywords: Resins, Sugar, dates, HPLC-MS, bioactive compounds.

الملخص: تعتبر التمور من أهم السلع الغذائية في دول الشرق الأوسط بما في ذلك سلطنة عمان حيث انها تعتبر غذاء صحي ومغذي للغاية. ومع ذلك فان هناك فاقد كبير، حيث يتم التخلص من مليوني طن من التمور سنويا في جميع أنحاء العالم. الهدف من هذه الدراسة هو إنشاء طريقة محسّنة لفصل السكريات عن التمر باستخدام الراتنجات الكروماتوجرافية ومعرفة تأثير هذه الراتنجات على المكونات الأخرى. وتم استخدام تمر الفرض كمثال للتمور في هذه الدراسة كما تم استخدام ثلاث راتنجات من النوع الهلامي قوي الكاتيون وهي د.ا.و–كالسيوم و ب.سي.ر–كالسيوم و ب.سي.ر–صوديوم. تم تحليل المستخلصات الناتجة من عملية فصل السكريات بجهاز الفصل الكروماتوجرافي السائل عالي الأداء المزود بكاشف مطياف الكتلة لقياس السكريات والبوليفينول. أظهرت النتائج امتصاص معظم السكريات بواسطة جميع الراتنجات المختبرة مع انتقائية أعلى تجاه الفركتوز مقارنة بالجلوكوز والسكروز. كان د.ا.و–كالسيوم أقل امتصاص للسكر مقارنة بالراتنجات الأخرى. كما كشف اختبار تحليل المعادن بواسطة جهاز البلازما عن انخفاض حاد في محتوى البوتاسيوم، وهو المعدن الرئيسي في تمور الفرض، كنتيجة لعملية الفصل. كما أظهر محتوى البوليفينول انخفاضًا حادًا بعد فصل السكر. بشكل عام نجح المشروع في تحديد طريقة لإزالة السكر من التمور وتحديد درجة امتصاص الراتنجات الكروماتوجرافية للمعادن والبولي فينول الموجودة في التمر. وللعمل المستقبلي يوصى بتقييم كفاءة راتنجات كروماتوجرافية أخرى في فصل السكر.

الكلمات المفتاحية: الراتنجات، السكر، التمور، جهاز الفصل الكروماتوجرافي السائل عالي الأداء المزود بكاشف مطياف الكتلة، المركبات الحيوية النشطة .

Introduction

One of the major concerns on dates' industry is the loss of freshly harvested dates during picking, storage, and processing stages (Parn et al., 2015). Approximately, two million tons per year of dates are abandon as wastes (Mrabet et al., 2017). Low-quality dates are not consumed by humans but rather discarded or combined in animal feed (Parn et al., 2015) due to their hard texture, insect infestation or fungus contamination (Abbès et al., 2011; Mrabet et al., 2017). The discarded dates was found to have similar characteristics compared with high grade dates especially in sugars, dietary fibers and safety (Besbes et al. 2009). Therefore, the low quality dates can be utilized into new products like biomass production or metabolites (Abbès et al. 2011). The gross economic loss in discarding low quality dates is believed to be much higher when the economic value of dates' bioactive compounds are considered (Elleuch et al., 2008). Bioactive compounds in dates waste or low-quality dates products could be extracted and used as value-added constituents. Therefore, high value-added products could be prepared from low-quality dates (Abbès et al., 2011). However, there are limited studies are reported on the dates' product development (Ahmad & Abu Bakar, 2018).

The main dates' product used in the food industry is molasses (i.e. syrup) (Bedeir, 2014). Dates' molasses can be consumed directly or as an ingredient in many food products (Abbès et al., 2011), such as jams, ice cream, bakery products, confectioneries and concentrated beverages (Abbès et al., 2011; Al-Mamary et al., 2014; Be-

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deir, 2014). The syrup is rich in essential nutrients and contains high sugar content which provides rapid energy to the human body (Abbès et al., 2011). The syrup consists mainly of moisture, fructose, glucose, and small amounts of sucrose, pectin, protein and calcium (Mostafazadeh et al., 2011). Dates' type (Mostafazadeh et al., 2011) and processing methods (Abbès et al., 2013) affect the date syrup components. Besides that, dates' syrup contains high amount of antioxidants that could reduce progression of many diseases, oxidative stress and inhibit macromolecules oxidation. Antioxidants such as phenolic acid, carotenoids, flavonoids, and ascorbic acid has been identified in dates (Abbès et al., 2013). The functional ingredients in dates that are biologically beneficial to human health (Bedeir, 2014) are greatly enhance dates' uses in nutraceutical businesses (Abu-Reidah et al., 2017). Additionally, phytochemicals substances enhance date fruit's organoleptic and nutritional properties (Baliga et al., 2011). Epidemiological studies demonstrated that routine intake of fruits and vegetables could decrease the risk of several chronic diseases due to their dietary antioxidants content (Abu-Reidah et al., 2017). Therefore, dates' syrup was recommended to be used as a functional food due to its biological components, e.g. antioxidants activity (Abbès et al., 2011). However, there is a concern of regular consumption of high amount of dates with high level of sugar, especially for non-communicable diseases (NCD) patient. Fortunately, with modern food technology, bioactive compounds in dates could be extracted individually or collectively.

On most dates, glucose and fructose are found in equal quantity whereas sucrose is at is lower level (Mostafazadeh et al., 2011). Due to the structural similarity of glucose and fructose, their isolation is difficult. There are several methods suggested to extract and purify sugars from sugar cane and beets including enzymatic breakdown, sugar-binding protein; lectins, fractional freezing techniques, and carbonation (Buchele, 2010). Each method has disadvantages such as high cost, undesirable breakdown, toxicity and strong bound complexes. Other studies had used toxic solvent to separate sugar from the media (e.g. juice), such as methanol, chloroform and acetonitrile. Also, there are chromatographic granular applied in the sugar refining process, which was found to be safe. Chromatographic carbohydrate separation mechanism is based on hydrophilic or hydrophobic interactions, ion exchange, size exclusion and ligand exchange (Gramblička & Polakovič, 2007; Nobre et al., 2009). The resins of sulfonated polystyrene-co-divinylbenzene (PS-DVB) are largely used in sugar industry due to their higher selectivity and capacity and they are chemically inactive (Luz et al., 2008).

Generally, resins are classified into two major groups; macroporous and gel-type. Resins are functionalized with cations to form complexes with sugar hydroxyl group and according to the orientation of this group a selective adsorption occur. Therefore, cation and sugar conformation limits the cation-sugar affinity and distribution coefficient. In addition, number of OH groups have a major effect on cation-sugar complex stabilities (Tiihonen et al., 2002). Moreover, the formed adsorption forces between resins and sugars are weak forces and thus are easily broken by hot water (Buchele, 2010). Calcium gel-type resins are the mostly used resins in food industry for fructose-glucose separation (Luz et al., 2008). Nevertheless, sodium and potassium cation resins are recommended for these sugar separation (Nobre et al., 2009).

Several patents were registered that documented the use of chromatographic resins in producing sugar-free juice (Blase & Thomas, 2008; Buchele, 2010; Pease and Pu, 2016). Baikenov et al. (2020) published a work describing the optimal parameters for the separation of glucose, fructose, and oligosaccharides from glucose-fructose syrup using chromatographic resins. In the case of dates, Mostafazadeh et al. (2011) implemented the same approach to separate sugars from date syrup.

However, all the aforementioned methods aimed to optimize and model sugar separation process without studying the effect on the other important compounds such as polyphenols and minerals. Therefore, the objective of this study was to study the efficiency of different chromatographic resins (calcium and sodium resins) in removing dates' sugars and their impact on other important compounds that exist in dates. The final aim was to produce date products.

Materials and Methods

Materials and Chemicals

Date sample of Fard variety in fully ripe stage (*Tamar* stage) was purchased from the local market. All reagents and standards were of analytical grade and were obtained from Sigma-Aldrich unless otherwise stated. Methanol LC-MS grade from Fisher Scientific (UK), Acetonitrile LC-MS grade from BDH Chemicals (Germany), nitric acid for trace metal analysis from BDH (Germany) and ammonium acetate HPLC grade from J. T. Baker (Germany). The resins used were Dowex™ MONOSPHERE™ 99Ca/320 (DOW-Ca) (DOW CHEMICAL, USA), PCR641Ca 3282Q/18/2 (PCR-Ca) and PCR642Na MR7-916 (PCR-Na) (Purolite®, USA). Specifications of the used resins are shown in Table1.

Table 1. Resins Characteristics

Resin	Type	Ionic form	Volume diameter	Functional group
DOW-Ca	Strong acid Cation, gel	Ca++	300 - 330 µm	Sulfonate
PCR-Na	Strong acid Cation, gel	Na+	315 ± 20 µm	Sulfonic acid
PCR-Ca	Strong acid Cation, gel	Ca++	310 ± 10 µm	Sulfonic acid

Sugar Separation

Sample Preparation: The chart in Figure 1 illustrates sample preparation steps for sugar extraction. Sugar extraction was done similarly to the method described by Myhara et al. (2000) with some modifications. Briefly, 100 g of pitted dates were homogenized with 70% or 75 or 80% (70 °C) ethanol in a ratio of 1:4 (w: v) by ultra-mixer homogenizer (Ultra-Turrax, T-25 basic) at a speed of 13,000 rpm till a homogeneous slurry was produced. Then, the solution was sonicated in a water bath at 70 °C for 15 minutes. After that, the solution was centrifuged at 10,000 rpm, at 30 °C for 10 min. The supernatant was collected and filtered using glass microfiber filter paper (A, Whatman) while the residue was extracted again by ethanol solution following the same procedure used above.

The extraction step was repeated thrice for complete removal of sugar. Only the first extract was used in the subsequent analysis. Then, the residue was dried in an oven (Carbolite, UK) at 85 °C for 6 h, milled and stored in a freezer (- 20°C) till further analysis. Sugar concentration of the filtered supernatant was measured by an automatic refractometer (HI 96802, HANNA Instruments Inc., Romania) then diluted by 70% or 75 or 80% ethanol to produce 10% and 15% ethanolic sugar solution. Summary of the sample preparation steps are shown in Figure 1.

Resin Conditioning: Three different types of resins were used for sugar extraction; DOW-Ca, PCR-Ca and PCR-Na. The resins were conditioned before their use as per the manufacturer instructions with minor modification. Briefly, the resin was soaked in deionized water in 1:4 (v/v) ratio for 5 min. Then, the water was decanted and 70% or 75% or 80% ethanol was added to the conditioned resin in a ration 1:4 (v/v) and left for 5 min to ensure complete water removal. Then the ethanolic solution was decanted.

Sugar Extraction: The diluted ethanolic sugar solution; 10% and 15% were sonicated for 5 min to remove gasses that could interfere sugar adsorption by resin as suggested by the resin manufacturer. Then, 50 ml of the conditioned resin was mixed with the diluted ethanolic sugar solution in different ratio (1:1, 1:2 and 1:3, resin: solution) in a reagent bottle. Two temperatures (20 °C and 40 °C) were used in this experiment that was adjusted using water bath. Sugar brix was measured by an automatic refractometer (HI 96802, HANNA Instruments Inc., Romania) at different time intervals; 1, 10, 20, 30 and 40 min. The mixture was caped and kept under continuous mixing using low speed in a magnetic stirrer plate throughout the experiment. After 40 min, the ethanolic sugar solution was decanted carefully from the resin and collected in a separate beaker. The resin was washed and conditioned before another cycle of extraction. An aliquot (5 ml) of the ethanolic sugar solution was taken for further analysis and the rest was subjected to another extraction cycle using the same resin after it is being washed and conditioned using the same procedure used in cycle 1. The various experiment parameters are summarized in Table 2.

Resin Washing: To release the adsorbed sugar from the resin before moving to the second extraction cycle; resins were washed using hot water as per the instructions of the manufacturer. Prior to the washing step, the resins were soaked in 100 ml de-ionized water (i.e. at room temperature, RT) for 5 min to remove any residual ethanol and then the water was discarded. Then, hot deionized water (70 °C) in a ratio 1:2 was added. The mixture was kept under continuous stirring using a magnetic stirrer in a hot plate. Brix was measured continuously till no further change in the reading; then water was removed and a new hot deionized water was added. The washing step was repeated three times. After washing, the resin was re-conditioned again by ethanol (70% or 75 or 80% depending on the starting ethanol solution) to remove any remaining water before starting a new extraction cycle.

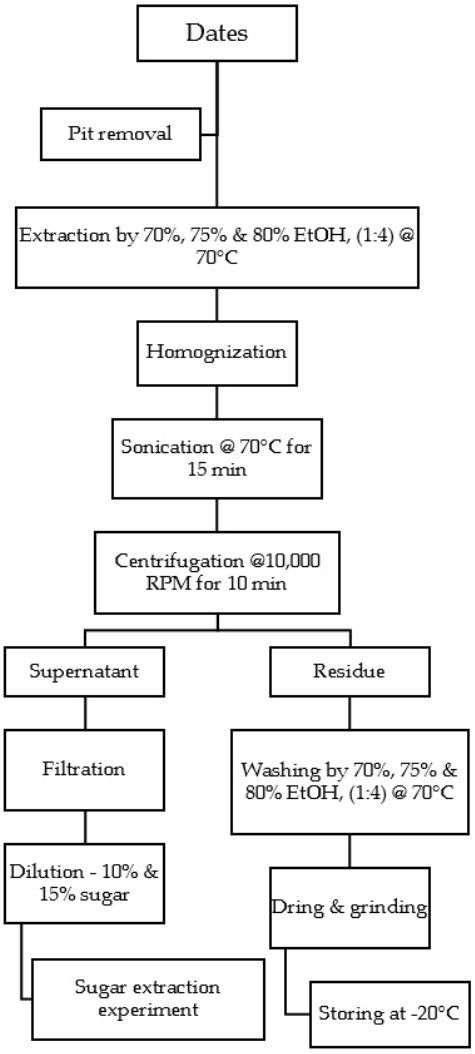

Figure 1. Summary chart of sample preparation

Table 2. Experimental parameters for sugar separation

Parameters	Conditions				
Sample sugar %	10%	15%			
Temperature	20oC	40oC			
Extraction cycle	1	2			
Resin	DOW-Ca	PCR-Ca	PCR-Na		
Solvent Conc.	70%	75%	80%		
Ratio (resin: sample)	1:1	1:2	1:3		
Time	1 min	10 min	20 min	30 min	40 min

Sample Concentration: Samples collected from first and second extraction cycles as well as from dates' stock solution were dried by vacuum concentrator (Concentrator plus, Eppendorf, USA) at 30 °C. Dried samples were stored at -60 °C until further analysis.

Ash Determination

Samples were ashed using a muffle furnace (CARBOLITE, UK) at 550 °C for 18 h (AOAC 2000, Method p). Few drops of hydrogen peroxide were added to samples that had carbon. Samples that showed persistent black spots were suspended in a deionized water and filtered through an ashless filter paper (Whatman No. 541). The filtrate was dried in an oven and the filter paper with the black materials were ashed using the same conditions used initially.

Minerals Profile by Inductively Coupled Plasma (ICP)

Sample Preparation: Some of the ashed samples were used to determine minerals profile by ICP, including date fruit, date residue, sugar extract and 15% (1:3) first cycle according to (EN act Oct1, 2017) method with slight modification. The ashed sample was digested by adding 2 ml concentrated nitric acid HNO_3 and kept in a hot plate at 250 °C until it was dissolved. Then drops of deionized water were added to cool the solution, thereafter, transferred to 10 ml volumetric flask and diluted with deionized water to the mark. After that, the solution was filtered using ashless filter paper (Whatman #541) and then stored in a fridge at 2 °C until it was injected to ICP.

ICP Conditions: Perkin Elmer-optima 8000 ICP-OES with spectral range 165-900 nm and resolution < 0.009 nm @ 200 nm was used. The detector was UV-sensitive, dual backside-illuminated Charge-Coupled Device (CCD) array detector with two photosensitive segments containing 176 by 128 pixels. The detector is cooled to -8 °C using a single-stage integrated Peltier cooler. The RF COIL power was 1500 W and the peristaltic pump flow rate was 1 ml/min. The plasma gas was argon with flow rate of 8 L/min and the shear gas was compressed air. The tested elements were arsenic, zinc, lead, cobalt, cadmium, nickel, iron, boron, mercury, manganese, chromium, copper, aluminum, sodium, potassium, magnesium and calcium.

Sugar and Polyphenol Quantification by HPLC-MS

Sugar and polyphenols were quantified using Nexera-X2 LC instrument (Shimadzu Corporation, Japan) composed of LC-30AD pump, SIL-30AC autosampler and mass-spectrometer LCMS-2020 following the manufacture recommendation. The column used was Imtakt Unison UK-Amino column (250×3 mm, 3 µm) maintained at 37 °C by the unit CTO-20AC. The ionization mode interface was DUIS (ESI and APCI) and DL temperature was 250 °C and heating block at 300 °C. Nitrogen was used as nebulizing gas and drying gas at a flow rate of 1.5 L/min and 15 L/min, respectively. Four events were used, two for scan (positive and negative) and the other remaining two events were SIM positive and negative according to m/z ± (H+) or + (CH_3COOH-). Interface voltage was 3.5 kV, DL voltage, quarry DC and RF voltage were sat at default. Scan speed was 10000 u/sec from 100 m/z until 1000 m/z with event time 0.1 s. The mobile phase consisted of LC-MS grade acetonitrile (mobile phase A) and 100 mM ammonium acetate (mobile phase B). The gradient for mobile phase B was programmed as follows, 5%-100% (0-15 min), then 100% for 5 min then 0% for 6 min. The concentrated samples were dissolved in 1 ml methanol and filtered into vials using 0.22 µm nylon syringe filter (ALWSCI Group, China). Sample injection volume was 1 µL and flow rate was 0.3 ml/min.

Statistical Analysis

The statistical analysis was carried out using Microsoft Excel 2021 (version 16.57,© 2021 Microsoft) to calculate one-way analysis of variance (ANOVA) to test significance of the means of the samples. The significance of the difference between the analyzed group parameters was analyzed by Student's t- test. Also, R program (version 4.1.1 (R Core Team, 2021)) used to test parameters correlations by Tukey test, Regression test and ANOVA interaction plots. $P<0.05$ was considered statistically significant. The collected data were expressed as mean ± standard deviation (SD).

Results

Optimization of Separation Condition

Many tests were performed before finalizing sample preparation and extraction procedure. In reference to

the sample preparation, deionized water was compared with ethanol to check the best solvent for sugar solubilization that will facilitate sugar adsorption by the resin. It is noteworthy that Mostafazadeh et al. (2011) used deionized water to solubilize dates' sugars. Also, mode of process was evaluated by comparing column (separatory funnel) to batch under vacuum (conical flask). The results (not shown) found that resins adsorbed more sugar (higher capacity) when ethanolic solution was used. Moreover, ethanol offered many other advantages such as lower solubility of minerals and precipitation of water-soluble polysaccharides such as pectin. With regard to mode of separation, batch separation was better than column extraction due to longer contact time between resin and sugar molecules. Vacuum was not critical factor on the experiment since the solution was degassed. Furthermore, use of refractometer to measure sugar content in ethanolic solutions was a challenge as ethanol itself gives a reading. This problem was solved by spiking the different ethanolic solutions (70%, 75%, 80%) with sugars to construct a calibration curve to normalize effect of ethanol on brix readings. Standards were prepared by dissolving glucose (10% w/w) and fructose (10% w/w) in the different ethanolic solutions to produce 20% stock ethanolic sugar solution. Then, the solution was diluted into several concentrations. The curve equation for each ethanolic solution (70%, 75%, 80%) was used to determine sugar concentration in all the solutions afterwards.

Date Sugar

In this study, glucose, fructose and sucrose were quantified in *Fard* dates using HPLC. The analysis was found to be the most important factor that affects resin chromatographic separation (Baikenov et al., 2020). The results of sugar content and types are shown in Figure 2. This information was used to adjust dates' sugar-ethanolic extract to the desired concentration. As per the obtained data, in 100 g of dates, glucose and fructose level was ~ 35 g individually whereas sucrose was ~1.6 g. Level of ethanol (70-85%) was not significant in sugar extraction from *Fard* fruit.

Parameters of the Separation Method

Interactions of The Method Parameters: The aim of this work was to find an effective method to separate sugars from dates' extract. Several factors and variables were tested to reach to the optimum separation conditions. These factors were adsorbent (resin) type, sugar concentration, solvent concentration, resin to sample ratio, extraction temperature, experiment time and number of extraction cycle. Through R program, the variations between these factors with respect to sugar concentration readings after each treatment were tested by Tukey multiple comparisons of means. Table 3 illustrates the results of this test for extraction cycle 1 and Table 4 for extraction cycle 2.

Interaction of experimental time and resin types in extraction cycle 1 and extraction cycle two showed no significant differences as it is clear in Figures 3 and 4. However, there was a significant difference (P <0.05) between sample to resin ratio (1:1, 1:2, 1:3) within time in extraction cycle 1 (Figure 5) and extraction cycle 2 (Figure 6). Secondly, temperature (temp) did not show an effect (20°C vs. 40°C) in sugar separation in the extraction cycle 1 (Figure 7). However, in extraction cycle 2 the time interval from 20 min to 40 min was signifi-

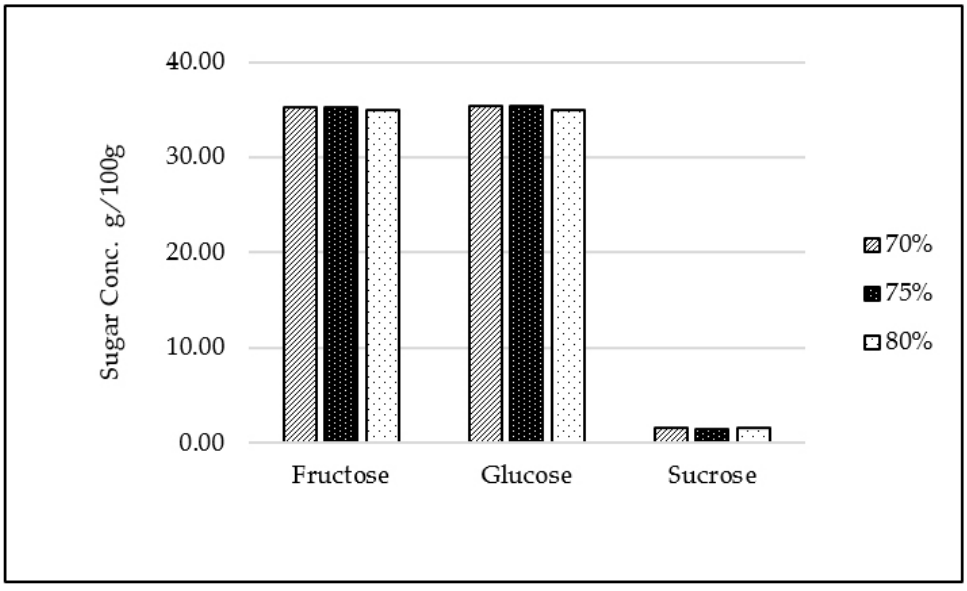

Figure 2. Effect of ethanol concentration on extraction of sugar from Fard dates.

Table 3. Analysis of variance for cycle 1

Parameter	*Df*	*Sum Sq*	*Mean Sq*	*F value*	*Pr(>F)*	
resin	2	4.4	2.2	1.990	0.138	
Temp.	1	0.0	0.0	0.005	0.944	
de.conc	1	1597.9	1597.9	1432.704	< 2e-16	***
ssratio	2	967.9	483.9	433.910	< 2e- 16	***
time.interval	4	1358.7	339.7	304.559	< 2e- 16	***
Residuals	529	590.0	1.1			

*de.conc: dates extract concentration (10 %& 15%), ssratio: solution: resin ratio, Signif. codes: '***' = 0.0*

Table 4. Analysis of Variance for cycle 2

Parameter	*Df*	*Sum Sq*	*Mean Sq*	*F value*	*Pr(>F)*	
resin	2	5.5	2.8	2.618	0.07391	
Temp.	1	13.0	13.0	12.305	0.00049	***
de.conc	1	435.4	435.4	411.591	< 2e-16	***
ssratio	2	2286.0	1143.0	1080.457	< 2e-16	***
time.interval	4	724.7	181.2	171.266	< 2e-16	***
Residuals	529	559.6	1.1			

*de.conc: dates extract concentration (10 %& 15%), ssratio: solution: resin ratio, Signif. codes: '***' = 0.0*

cantly different (P <0.05) in brix readings between 20°C and 40°C (Figure 8). Moreover, there was a significant difference (P <0.05) between the two tested concentrations (15% and 10%) according to brix readings with experiment time interval in extraction cycle 1 (Figure 9) and extraction cycle 2 (Figure 10).

Resin-Sugar Holding Capacity: Due to the differences in the functional group and cation in the used resin, holding capacity was different as illustrated in Figures 11-13. PCR-Ca was the highest sugar adsorbent (99%) followed by PCR-Na, however, the differences between the two resins was not significantly different (P<0.05). Dow-Ca resin showed the lowest adsorption capacity towards sugar; maximum adsorption was towards fructose (94%). As it's clear from Figures 11-13, sugar holding capacity is proportional with resin:solution ratio. Also, 80% ethanol concentration enhanced sugar absorptivity by resin compared to the other concentrations (70% and 75%).

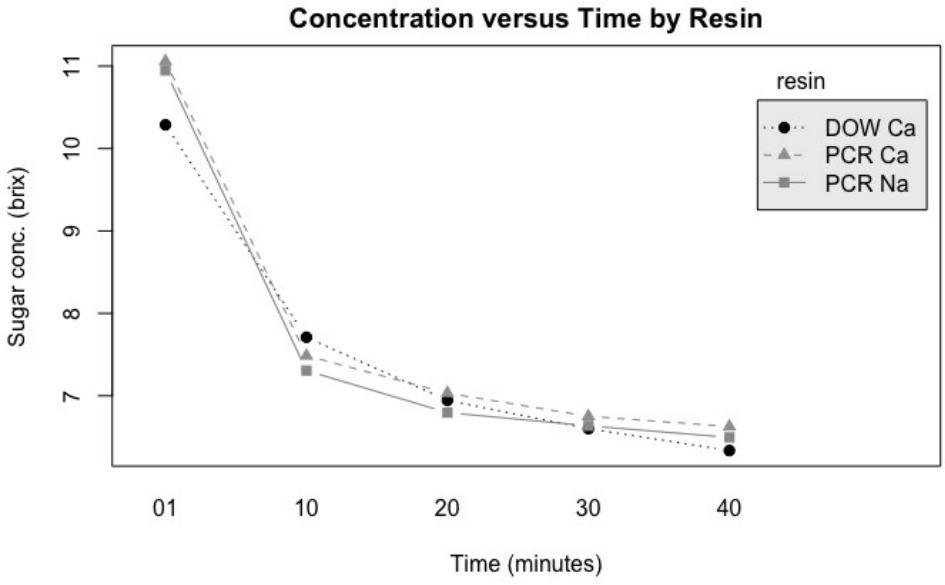

Figure 3. Correlation between resin types and time in cycle one

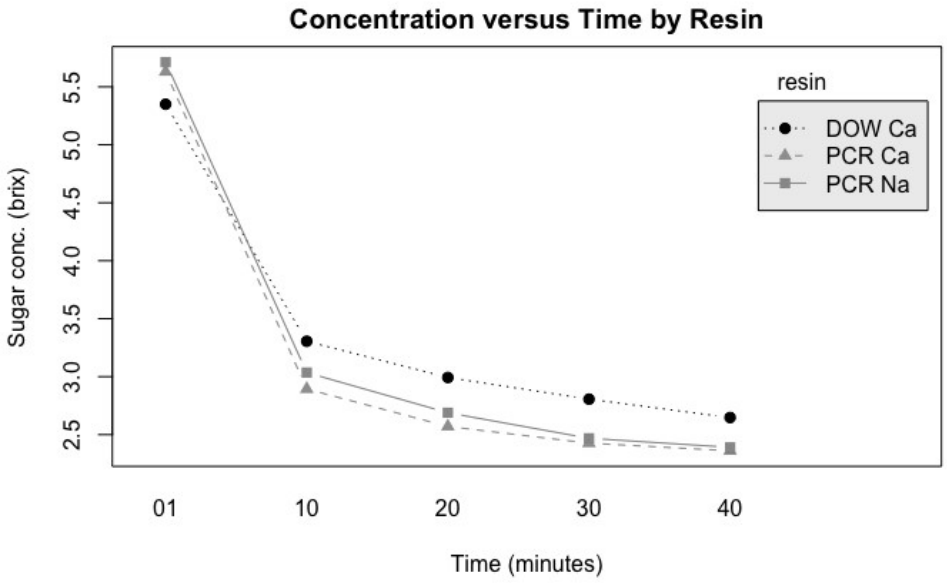

Figure 4. Correlation between resin types and time in cycle two

Resin-Sugar Affinity: All of the tested resins adsorbed most of the sugars in the sample, especially PCR-Na and PCR-Ca compared to DOW-Ca. However, fructose was adsorbed more than both glucose and sucrose.

Resin Washing: As mentioned earlier, sugar separation in this study was based on an adsorption cycle followed by a desorption cycle (Nobre et al., 2009). In the desorption cycle, sugars separated from resin by washing using hot water. According to the washing procedure, it involved three cycles of washing by hot deionized water. In each cycle, brix was measured to check efficiency of the desorption process. The sum of Brix readings for each washing cycle is illustrated in Figure 14. At washing cycle 1, most of the adsorbed sugars was released, > 60% of the total adsorbed sugar. DOW-Ca resins discharged the highest amount of sugars followed by PCR-Na then PCR-Ca. In cycle three, all of the tested samples recorded less than 1 g/ml of sugar. This makes the resin reusable for further sugar removal cycles.

Sugar Content

Glucose, fructose and sucrose were determined in the resin-treated ethanolic solution. The main findings are illustrated in Figure 15. HPLC-MS results were supporting

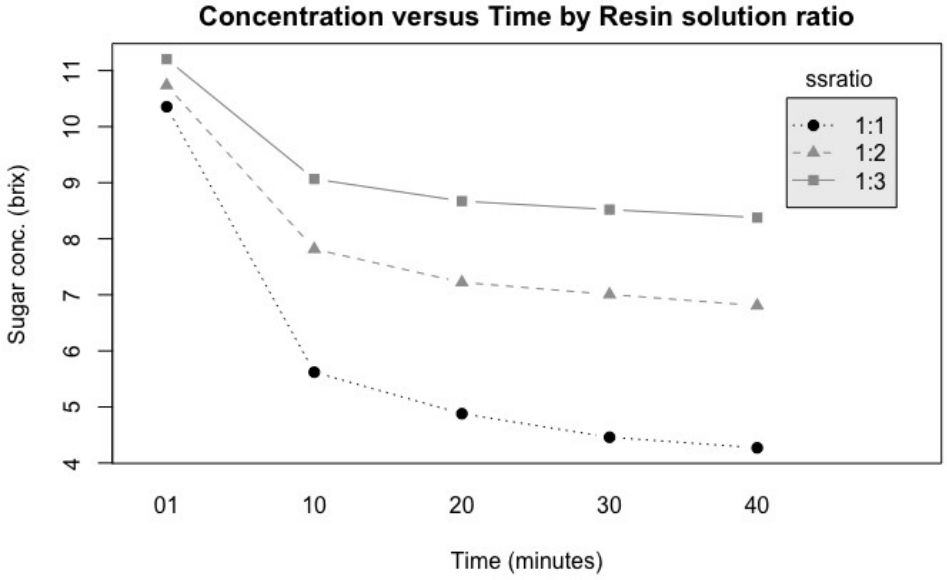

Figure 5. Dates solution ratios with time in cycle 1

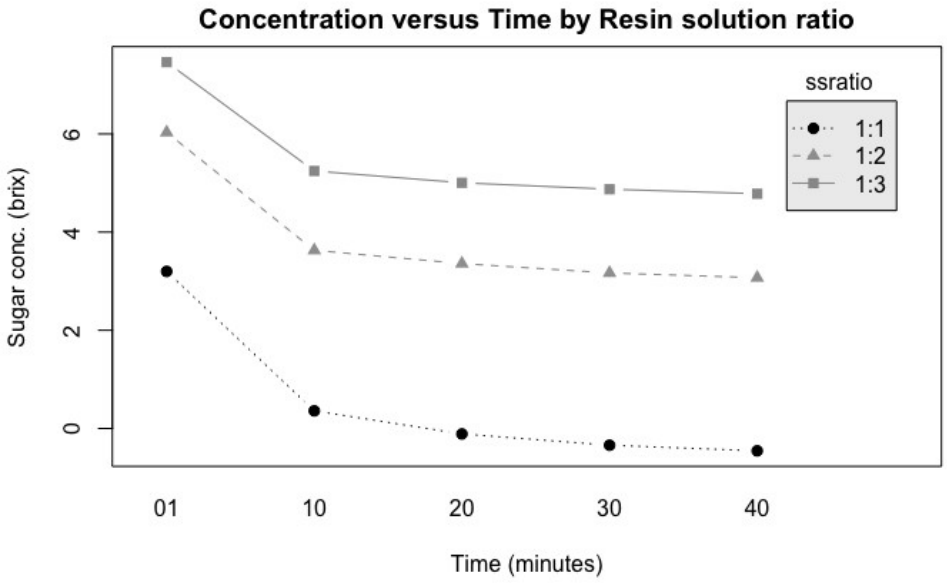

Figure 6. Dates solution ratios with time in cycle 2

brix readings at the separation experiment. From Figure 15 we can conclude that Ca resins, DOW- and PCR-Ca, were more selective to fructose than glucose and sucrose. Similar observation was also reported by Baikenov1 et al. (2020). While Na-resin (PCR-Na) had no sugar selectivity towards any of the sugars (i.e. glucose and fructose). Thus, calcium resins made the solution rich in glucose than sucrose and fructose. However, Na-resin produced sucrose rich solution. Overall, sugar content in the resulting extract from exposure to the various types of resins was very low compared to the original sugar content in dates. This illustrates that the experiment succeeded in removing sugars from dates' ethanolic extract.

Ash Content and Minerals Profile

Ash contents: All three used resins in this study adsorbed most of the ash during the first extraction cycle (about 98%). Figure 16 illustrates the main minerals in the 75% ethanolic solution extract after treatment with the different resins (1:3 resin to sample ratio). Resins with the same cation have similar properties; DOW- and PCR-Ca. Calcium became the main mineral followed by sodium and potassium in solutions treated by DOW- and PCR-Ca resins. However, sodium content was not affected in the solution treated by PCR-Na resin.

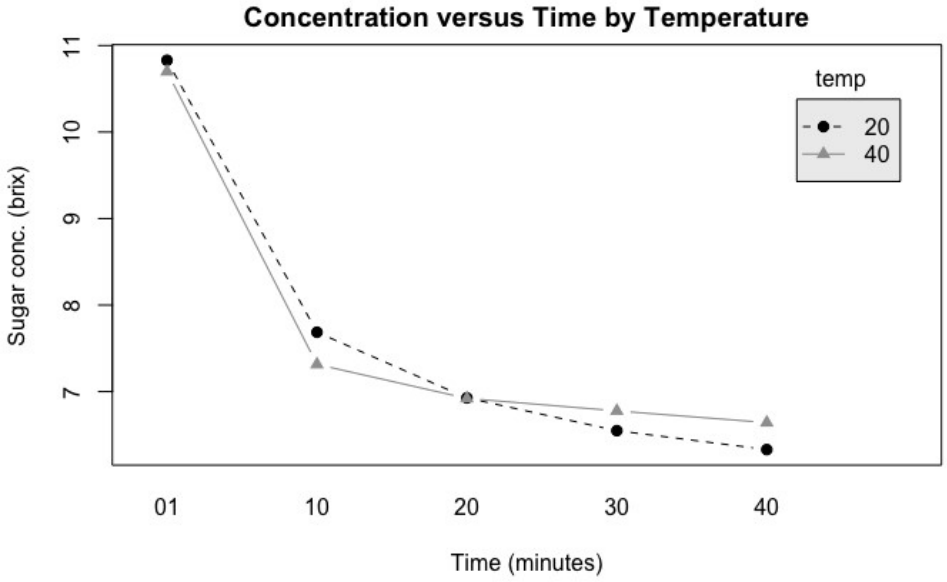

Figure 7. Temperature effect in brix reading with time in cycle 1

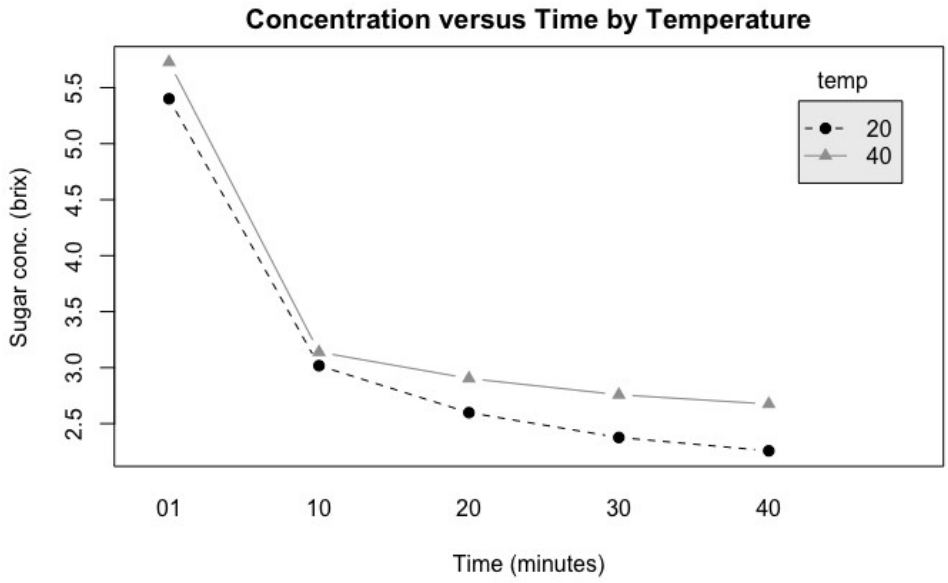

Figure 8. Temperature effect in brix reading with time in cycle 2

Polyphenol contents: Dates fruits are rich in polyphenols content as many studies proved. In this study, the free polyphenols content was determined using LC-MS. Total of 18 compounds were detected in *Fard* date samples as shown in Figure 17. Caffeic acid and the gallic acid were the dominant polyphenols.

Similar to the observation with whole *Fard* dates fruits, 18 free polyphenols were identified in the treated extract but with different proportions. This is because the resins had adsorbed some of the polyphenols. Polyphenols' adsorption varied with the resin type as shown in Figure18. Figure 18 displays the profile of polyphenols in the sugar ethanolic solutions resulted from treatment by the three different resins. It is very evident that PCR-Na and PCR-Ca resins adsorbed mainly sugars along with other compounds including free polyphenols. The percentage range of polyphenols hold by PCR-Na and PCR-Ca were 75% - 100% and 89% - 100%, respectively, depending on the type of the polyphenol. For example, m-coumaric acid was the lowest polyphenol adsorbed by PCR resins, whereas DOW-Ca resin adsorbed 95% of *m*-coumaric acid present initially in the solution.

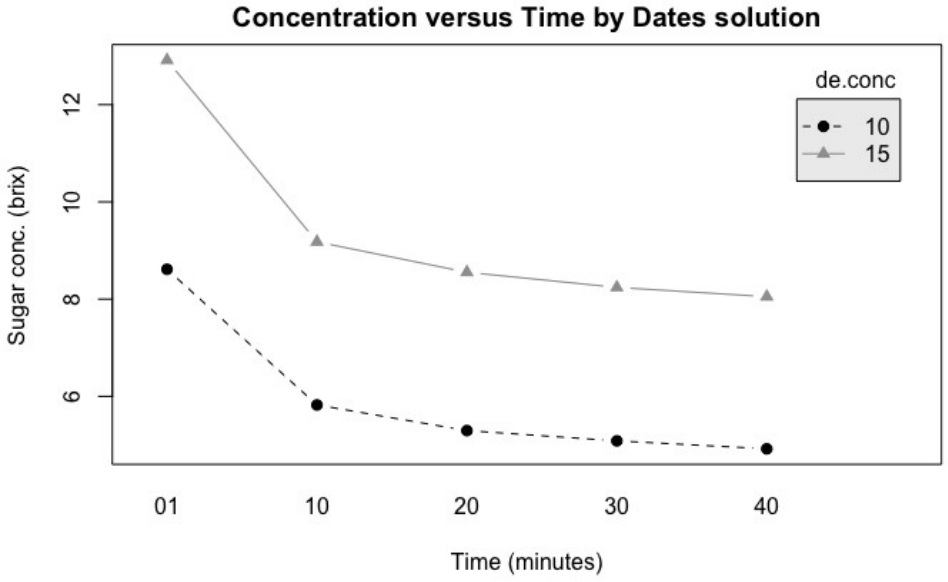

Figure 9. Sample concentration with time in cycle 1

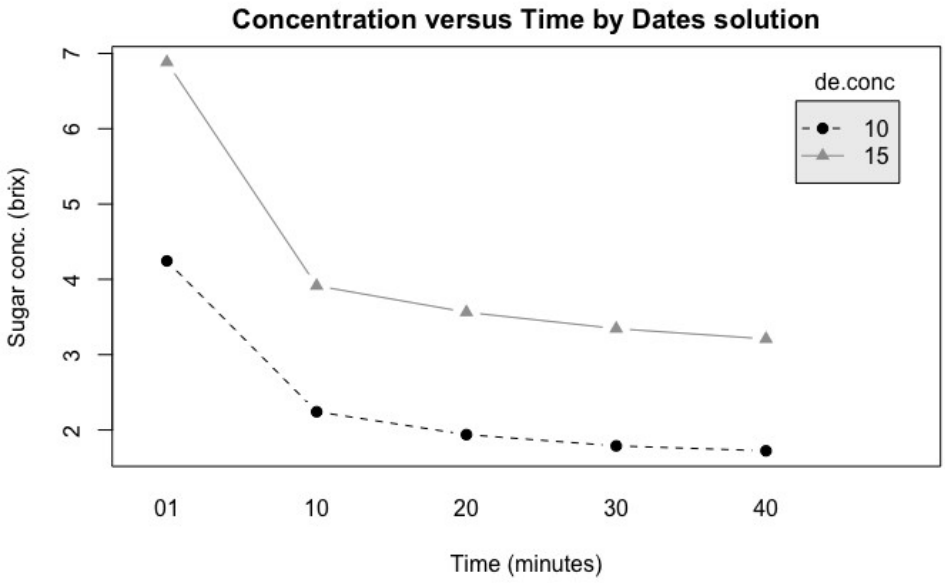

Figure 10. Sample concentration with time in cycle 2

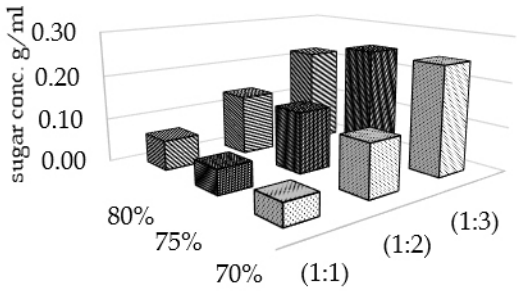

Figure 11. DOW 99 Ca sugar holding capacity

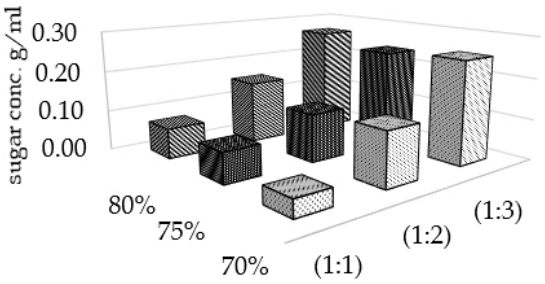

Figure 12. PCR642 Na sugar holding capacity

Discussion

Carbohydrates are the major component in dates fruit; around 70%-80% (DW) (Bedeir, 2014). Glucose and fructose are the main simple reducing sugars in dates along with the disaccharide sucrose (Al-Harrasi et al., 2014). Al-Farsi et al. (2005) reported that *Fard* dates contain 28 g/100g (FW) glucose and the same for fructose while the total sugar was 56.7 g/100g (FW). However, for the same variety, Myhara et al. (1999) found that 100 g (DW) contains 43.6 g glucose, 42 g fructose and 0.53 g sucrose. Al-Harrasi et al., (2014) determined the total carbohydrates in *Fard* dates which were 79 g/100 g (DW). The differences are attributed to moisture content, sample preparation and quantification methods. In this study, *Fard* dates was found to contain ~82% sugars which is in line with what was reported earlier for the same variety (Myhara et al., 1999). Furthermore, as it is clear in the Figures 2 and 3, there was no significant differences in the extraction power of glucose or fructose or sucrose content ($P < 0.05$) by different ethanol concentration (70%, 75% and 80%). This means either solution can be used for this step (sugar extraction step) to prepare sugar ethanolic solution. Sugars exhibit high solubility in ethanolic solution and the percentage of solubilization decreases with the decrease in water percentage (Bockstanz et al., 1989). Ethanol offers many advantages over other organic solvents, such as safety and re-usability while eliminates extraction of other ingredients (such as pectin) that could complicate the sub-

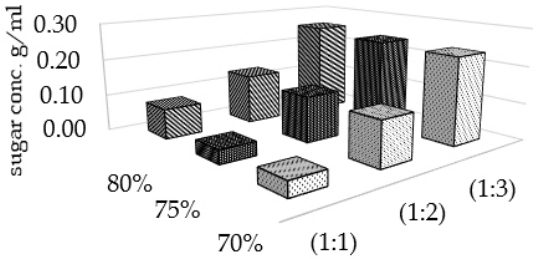

Figure 13. PCR641 Ca sugar holding capacity

sequent treatments (Rayo-Mendez et al., 2019). During resin treatment, ethanol was unfavorable medium for sugars, thus sugar migrated into the polar resins. Other tested parameters were significantly (P >0.05) affected in sugar adsorption at extraction cycle 1, except type of resin and temperature. However, in extraction cycle 2, resin type and waiting time (20-40 min) did not affect the separation significantly. A previous study employed Na^+ and K^+ cation resins for similar separation at 25 °C and 40 °C concluded that in mono-component sugar mixture higher temperature decreased adsorption while sugar adsorption increased with increased temperature in multi-component sugar mixtures (Nobre et al., 2009). Overall, the tested parameters had different effect on sugar adsorption. Temperature and the used resin types were not significantly effective variables in sugar adsorption. While sample concentration and resin:sample ratio were significantly effective (P >0.05) in sugar extraction within the different variables used.

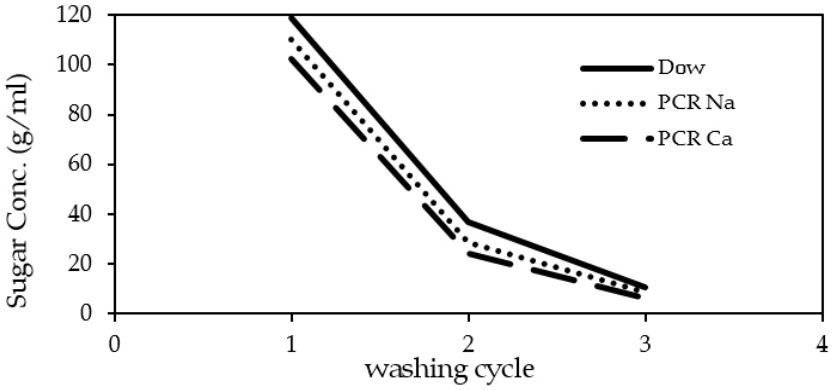

Figure 14. Sugar concentration in each washing cycle for each resin

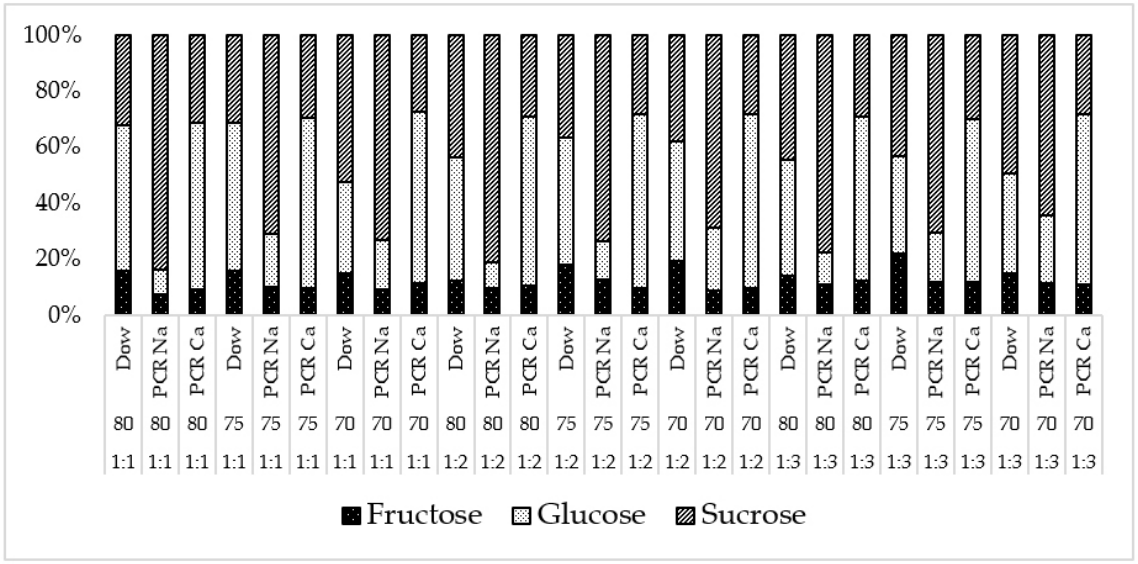

Figure 15. Fructose, glucose and sucrose ratio in sugar-free dates extract

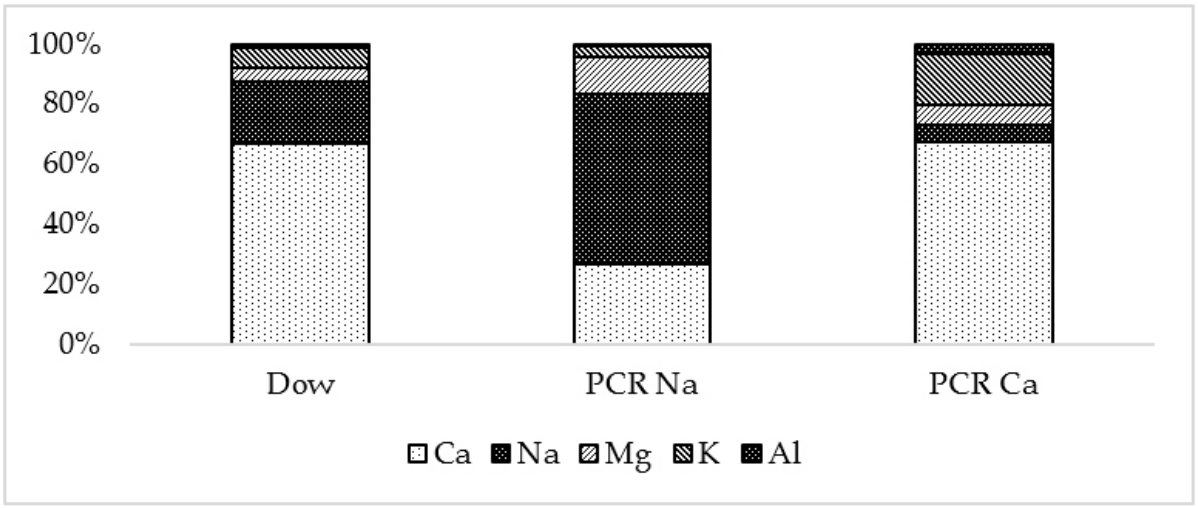

Figure 16. Main minerals in 75% ethanolic solution extract after treatment with 1:3 resin:sample ration

PCR-Ca was the highest in adsorbing sugar (99%) with no significant differences (P <0.05) within sugar type. However, DOW-Ca resin was the lowest in sugar adsorption. Cation type had effect in sugar adsorption and affinity. As proved by this study, the solution treated by calcium resins contained more glucose than sucrose and fructose. However, Na-resin-treated-solution contained higher sucrose. This result is supported by Mostafazadeh et al. (2011) findings where they used PCR-Ca to separate fructose from date syrup. Moreover, this finding was also observed elsewhere with other resins; potassium gel-type and sodium macroporous resins (Nobre et al., 2009).

Additionally, sugar selectivity was found to be unaffected by temperature. Work done by Nobre et al. (2009) on Na resins reported similar results to our findings. This was attributed to the similarity in molecular weight of sugars. However, fructose/sucrose selectivity was higher anticipated to fructose-resins strong adsorption. Also, gel-type resins are higher selective than macroporous resin towards fructose (Gramblička & Polakovič, 2007; Nobre et al., 2009). Moreover, Tiihonen et al. (2002) showed that higher ethanol content are better suited for sugar separation by Ca^{2+} column than Na^{+} and La^{3+} columns. Whereas water sugar mixture is best for La^{3+} columns.

The main mineral in *Fard* dates was potassium which had declined after exposure to the three tested resins. Also, the main free polyphenol in *Fard* dates fruits were caffeic and gallic acids. This finding is somehow different than other studies that reported gallic acid the main free polyphenol in Omani dates (Ahmed et al., 2013; Al Harthi et al., 2015). However, after exposure to the resin, rutin

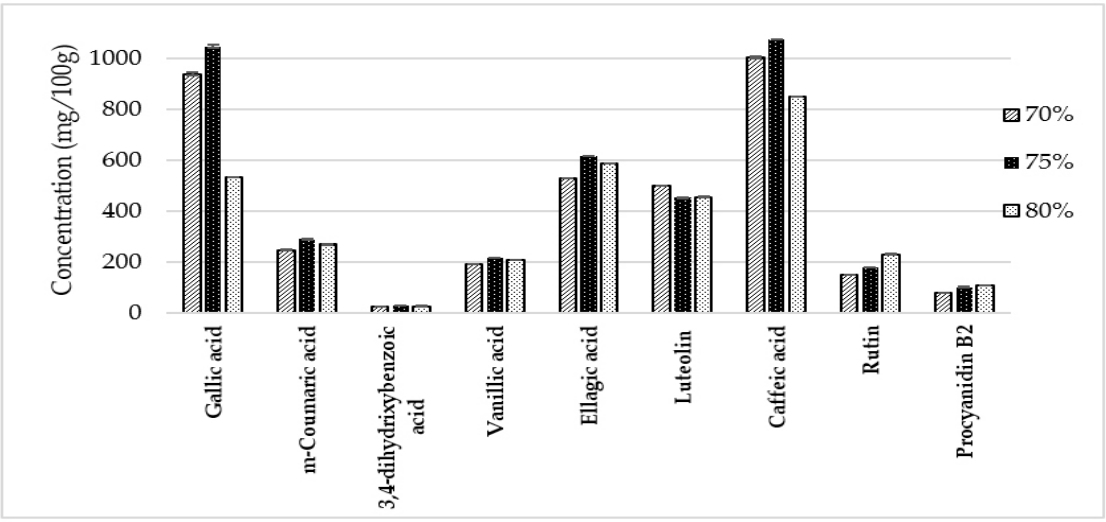

Figure 17. Polyphenols compounds extracted by different ethanol concentration from *Fard* dates

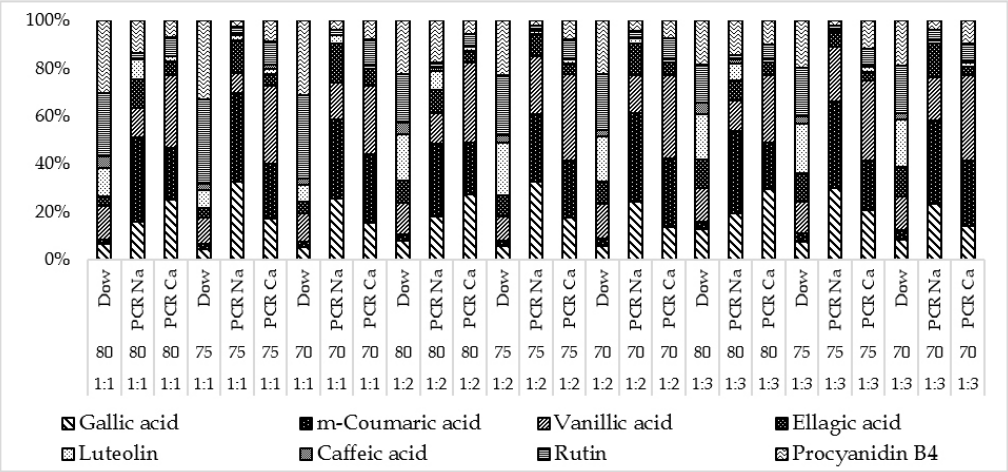

Figure 18. The main polyphenols (%) in resin treated sugar-ethanolic solution

and procyanidin B2 became the highest in the samples treated by DOW-Ca resin. For samples treated by PCR resins, m-coumaric acid and vanillic acid became the highest in PCR-Na and PCR-Ca treatments, respectively.

Conclusion

The method presented in this work showed that resins had different power and selectivity towards different sugars. PCR-Ca had the highest adsorbing power towards sugar (99%) followed by PCR-Na and DOW-Ca, respectively. Calcium based resins had more affinity towards glucose, whereas sodium based resin had more affinity towards fructose. Furthermore, all resins adsorbed minerals and polyphenols along with sugars. Temperature was found to be not critical.

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