HPLC-RID Sugar Content Analysis of Date Juice and Processed Products from Low Quality Thai Cultivar Date Palm (*Phoenix dactylifera* cv. KL1)

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Abstract

Thai Cultivar Date Palm (KL1) (Phoenix dactylifera cv. KL1) has been grown in Thailand for decades. Mature fruits are popular for fresh consumption. However, 50% or more of the fruit production was thrown away each year because of its low quality. 2) Objectives: To help farmers avoid wasting huge amounts of money, these low-quality fruits were preliminary developed into three value-added products. The fresh juice and its processed products were analyzed for types and amounts of sugars. 3) Methodology: The ripened date fruit was boiled and filtered to yield "boiled juice.". The juice was sterilized further using the retort technique under high temperature and pressure and called "retort juice.". The date-boiled juice was also concentrated to yield "syrup.". The type and amount of sugars contained in date palm fruit and its developed products were analyzed by a validated HPLC-RI detector. The injection volume of 3 µL was performed at 30°C with a flow rate of 1.4 mL/min using 75 % acetonitrile with 25% water as a mobile phase. The retention times of the containing sugars were compared with standards. 4) Result and Discussion: Thai Cultivar Date Palm (KL1) and its products contained higher amounts of monosaccharides than disaccharides. The mean values of fructose, glucose, and sucrose were in the range of 0.1449 – 1.4227 g/100 mL, 0.2539 – 1.6696 g/100mL, and 0.0893 – 0.3019 g/100mL, respectively. Galactose and maltose could not be detected. Among the products, syrup contained the highest amount of total sugar, followed by the retort juice, the boiled juice, and the fresh date juice, in order from highest to lowest in sugar content. 5) Conclusions: The waste date fruit could be developed into other value-added products. These products are a source of fast energy drinks, due to the high amount of monosaccharides that, are easier to absorb into gastrointestinal cells than disaccharides. The sugar contents increased significantly by temperature and time of heating. This paper is the first report on sugar analysis of Thai Cultivar Date Palm (KL1).

Keywords*: *High-performance liquid Chromatography, Refractive index detector, Fructose, Glucose, and Sucrose

1. Introduction

Date palm (*Phoenix dactylifera*) has been popular in countries across the Arabian Peninsula, North Africa, and the Middle East for a thousand years. Date palm cultivation has spread from its center of origin to the rest of the world since ancient times but only very recently to Thailand (Jaradat, 2011). More than 200 cultivars have been cultivated globally such as Bahee, Khalas, Khunaizi, Naghal, Khasab, Fardh, and Mabsli. Each cultivar is suitable for either fresh or dried consumption. *Phoenix* palms are dioecious, denoting that the trees are either male or female trees. They are a natural and highly nutritious source of dietary fiber (DF), acidic amino acids, essential minerals, vitamins, and sugars, especially monosaccharides (Al-Farsi and Lee, 2008). Sugar contents differ by cultivars and fruit maturation (Maqsood *et al*., 2020) ranging from about 40% (fresh dates) to 80% (dried dates), which mainly are glucose and fructose (Siddiq and Greiby, 2013). In a study carried out by Zhang *et al*. (2015), the total sugar content of 29 dried date

fruit varieties was quantified by HPLC. The monosaccharides content ranged from 61.7% to 78.6%. Among these, only Deglet Noor, Sukkari Al Qassim, and Nabtat Ali date contained a small amount of sucrose, while the rest of the varieties showed higher levels of fructose than glucose and were devoid of sucrose. As well as being eaten as fresh fruit, dates can be made into many processed products such as beverages, candy, chocolate, or syrup ("Silan" in Arabic). The sugar profiles of different stages of Barhee date fruit juices were studied by Burapalit *et al*., 2020. They revealed that dried dates contained three times more total sugar (69.42 g/100 g dry weight) than fresh dates (22.16 g/100 g dry weight) and premature dates (29.28g/100 g dry weight), while neither lactose nor maltose was detected in the fruits. In another study, Deglet Nour date syrup was analyzed using high-performance anion-exchange chromatography (HPAEC) with pulsed amperometric detection (PAD). The syrup contained equal amounts of glucose and fructose (36.4%, 36.5%) (Lajnef, *et al*., 2021). This high amount of monosaccharides in date fruits and their products show their potential benefits as a form of energy booster. Unlike disaccharides, these simple sugars do not need to be hydrolyzed before absorption through gastrointestinal cells. Hence, Muslim people can consume date products during Ramadan. Furthermore, date fruits and products have been shown to exhibit various medicinal properties such as galactagogue, antimicrobial, antioxidant, anticancer, antidiabetic, and antihyperlipidemic activities (Idowu *et al*., 2020).

In Thailand, many date palm cultivars have been imported and become an important commercial plant since 2002. However, their sensitivity to Thailand's climate, pests, and diseases required significant investment for cultivation, resulting in a high market price. Ten years later, the first Thai cultivar (*P. dactylifera* cv. KL1), was developed from the breeding of Deglet Nour and Barhee cultivars. The mature fruits vary in color from yellow, orange to red. The fresh fruit is sweet, juicy, and crunchy but has some astringency and bitterness. This cultivar has become more popular among Thai farmers since then because of the reduced cost of management. They are widely cultivated in the northern and north eastern regions, can adapt to a humid climate, flower in three-five years, produce large fresh fruits and give a high yield per crop (Intha and Chaiprasar, 2020). These locally grown cultivars are two or three times cheaper than imported varieties, making them more attractive to customers. However, Thai farmers lost a huge amount of money every year because some of the fruits were not good enough to sell, being too astringent or too small. Therefore, there has been a need to develop these low-quality date palms into other value-added products. In this study, the low-quality Thai cultivar date palm (KL-1) was preliminary developed into boiled juice, retort juice, and syrup. Then, the sugar types and contents of the fresh date extract and other date products were further investigated. This study will almost certainly be the first investigation of the sugar component of the Thai cultivar date palm (KL1) and its developed products. Furthermore, this data could help to establish and verify the health benefits of date palm by providing safety information on daily consumption for consumers.

2. Objectives

1) to analyze for types and amount of sugars of KL1 date palm juice and its products.

3. Materials and Methods

3.1 Material Preparation

The low-quality fresh and ripen date fruits (*P. dactylifera* cv. KL1) were generously provided by Aud date palm farm in Chaiprakarn, Chiang Mai, Thailand, between June and September 2020. The fresh dates were washed through tap water and air-dried before keeping in zip lock bags at -20 °C until use. The dates were divided into 4 groups and proceeded by different methods in the following.

3.1.1 Fresh juice: 500 g of fresh date fruit (without seeds) were mixed with 1 L of distilled water, then ground with an electronic blender. The filtrate was filtered through a muslin cloth. The fresh date juice was kept in Eppendorf at -20 °C until use.

3.1.2 Boiled juice: Ripe fresh dates were added with distilled water (1:1 ratio) and then boiled on a hot plate at 130 °C for an hour. The filtrate was filtered through a muslin cloth and kept in a glass bottle at -20 °C until use.

3.1.3 Retort juice: The boiled juice from 3.1.2 was further retorted at 118 °C, under pressure at 1.8 bar for 15 min to sterilize. The juice can be kept longer for at least one year at room temperature.

3.1.4 Syrup: The boiled juice from 3.1.2 was slowly stirred on a hot plate until viscous. The syrup was cooled down and kept in the glass bottles at room temperature until use.

3.2 Reagents

Standard sugars i.e. fructose, glucose, galactose, sucrose, and maltose were purchased from Sigma Aldrich (St. Louis, MO, USA). All HPLC grade solvents were purchased from Merck KGaA (Darmstadt, Germany). The deionized (DI) water was purchased from RCI Labscan Ltd. (Bangkok, Thailand).

3.3 Standard Solution and Sample Preparation

One gram of each standard sugar was dissolved in 50 mL of DI water, filtered through 0.22 µm, 13 mm, nylon membrane filter, and then diluted in various five concentrations. Each date sample (3 g) was mixed with Acetonitrile 25 mL and adjusted to 100 mL with DI water. The mixture was centrifuged to get rid of undissolved materials at 600 rpm and filtered through a 0.22 µm, 13 mm, nylon membrane filter in an HPLC vial.

3.4 System Validation and Quantification of sugars in date fruits

3.4.1 Linearity

Linearity was established by triplicate injections of five different concentrations of the standards dissolved in DI water i.e., fructose (2.1 to 21.1 mg/mL); glucose (2.2 to 22.7 mg/mL); galactose (2.0 to 21.0 mg/mL), sucrose (2.0 to 21.0 mg/mL) and maltose (2.0 to 20.3 mg/mL). The calibration curve for each sugar was obtained by plotting the concentration of the compound versus the area of the respective peaks [Figure 1].

3.4.2 Precision

The repeatability of the method was assessed by determining the coefficient of variation (CV%) of the areas obtained from the injection of 3 replicates of the standard solutions (fructose, 0.5 mg/mL, glucose, 0.5 mg/ mL, galactose, 0.5 mg/mL, sucrose, 0.5 mg/mL and maltose, 0.5 mg/mL) and the date palm products added to this mixture, respectively. In the evaluation of intermediate precision, three replicates were used over three non-consecutive days.

3.4.3 Accuracy

Accuracy was calculated by considering the recovery obtained for each compound at different concentration levels. Each sugar at 0.1 mg/mL were added 3 concentrations of each sugar i.e., 0.1, 0.2 and 0.4 mg/mL. The results were expressed by %recovery.

3.4.4 Sensitivity

The standard deviation and slope of the calibration curve were considered for a limit of detection (LOD) and limit of quantification (LOQ). The LOD and LOQ were determined with the formula 3.3α/S and 10α/S, respectively. Where α is the standard deviation of the y-intercept and S is the slope of calibration curve.

3.4.5 Quantification of sugar

The standards and samples were performed by HPLC coupled with the refractive index detector (Agilent 1100 G1362A Refractive Index Detector, Agilent Technologies, Inc., Santa Clara, CA, USA).

Analysis was acquired using the ZORBAX carbohydrate analysis column (4.6 mm ID x 150 mm; 5 µm). The injection volume was 3 µL and performed with an isocratic system of 75 % Acetonitrile with 25% water, a flow rate of 1.4 mL/min at 30°C for 15 min. The HPLC system was validated by linearity, precision, accuracy, and sensitivity.

3.4.6 Statistical analysis

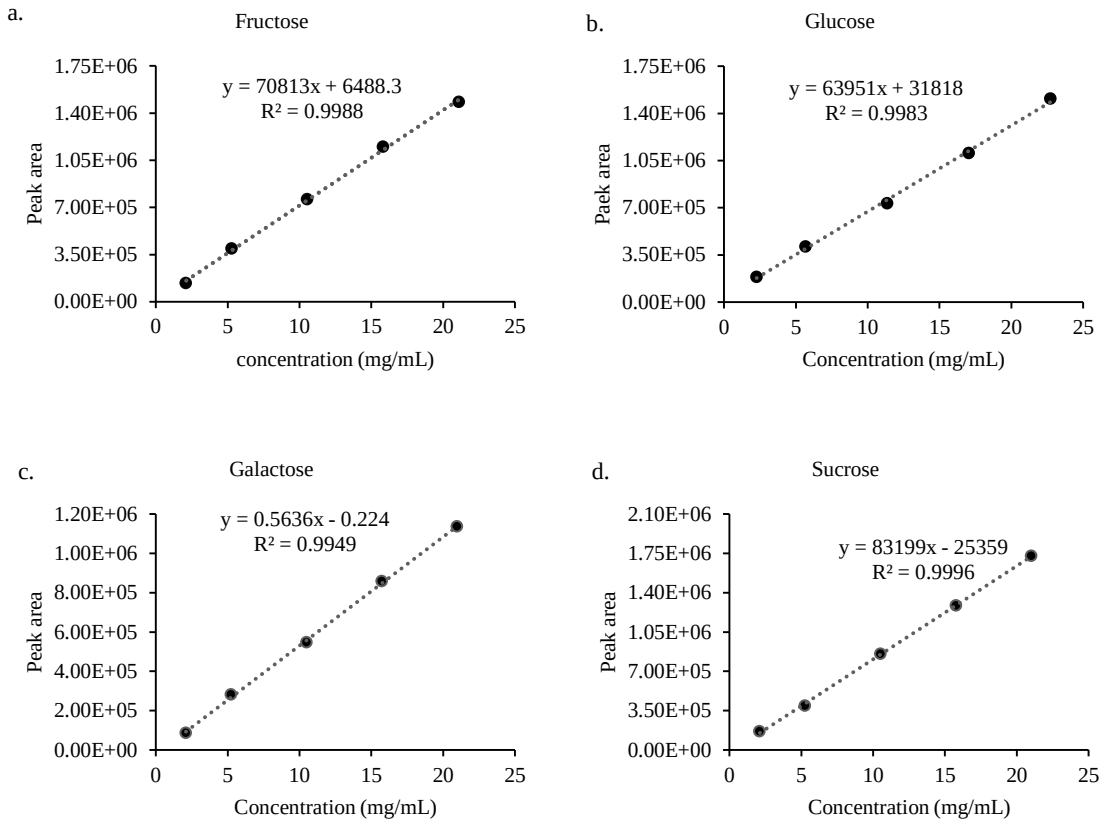
Statistical significance was calculated using Duncan's test comparing the means of each sugar between samples. Values are expressed as mean ± SD of multiple replicates. Bars labeled with different letters are significantly different ($P<0.01$).

4. Results and Discussion

4.1 Results

Chromatographic Conditions

The analytical method validation was performed as ICH guideline method validation. All validation factors were in the range of standard limitations [Table 1]. Regression equations of all standard sugars were linear with the coefficient of determination (R^2) in the range of 0.9949 - 0.9996 [Figure 1]. The method has high repeatability with high recovery values (99.40 – 106.3%). The relative standard deviation of all the parameters intra-day was 1.03 – 1.32% and inter-day was 1.14 - 1.35%, which was less than 2.0%, confirming that the method was precise. LOD and LOQ were lower than the amount of each sugar found in the samples. Therefore, this HPLC method is regarded as selective, accurate, and precise for sugar quantification.



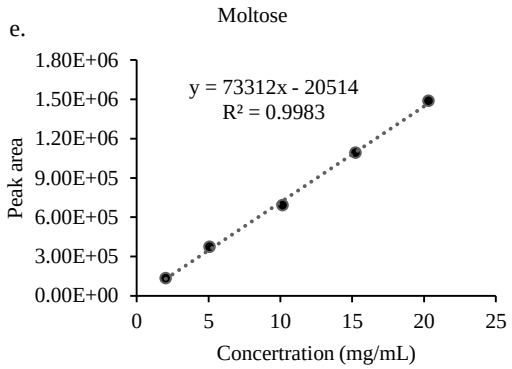


Figure 1 The standard curves of all five standard sugars, fructose (a), glucose (b), galactose (c), sucrose (d), and maltose (e), the regression equations of all standard sugars were linear with the coefficient of determination (R^2) in the range of 0.9949 - 0.9996, the analysis was performed in three replicates.

The selectivity of the compounds was determined by the correlation of the retention times (R_t) obtained from the five standard sugars. HPLC chromatogram and the retention time (min) of fructose (3.966), glucose (4.533), galactose (4.954), sucrose (6.342), and maltose (7.885) show in Figure 2.

Table 1 Validation parameters of HPLC-RID for analysis of sugars

Standards	Validation parameters				
	intraday precision (%)	interday precision (%)	% Recovery	LOD	LOQ
Fructose	1.21 ± 0.12	1.25 ± 0.02	101.2 ± 0.12 105.3 ± 0.24 99.7 ± 0.02	0.01	0.03
Glucose	1.27 ± 0.20	1.30 ± 0.01	99.5 ± 0.25 103.2 ± 0.14 106.3 ± 0.08	0.01	0.03
Galactose	1.32 ± 0.18	1.35 ± 0.07	102.4 ± 0.09 103.2 ± 0.10 105.2 ± 0.31	0.01	0.03
Sucrose	1.18 ± 0.05	1.19 ± 0.11	99.4 ± 0.17 99.8 ± 0.04 101.7 ± 0.03	0.02	0.05
Maltose	1.03 ± 0.03	1.14 ± 0.08	100.3 ± 0.09 104.8 ± 0.12 103.5 ± 0.16	0.02	0.05

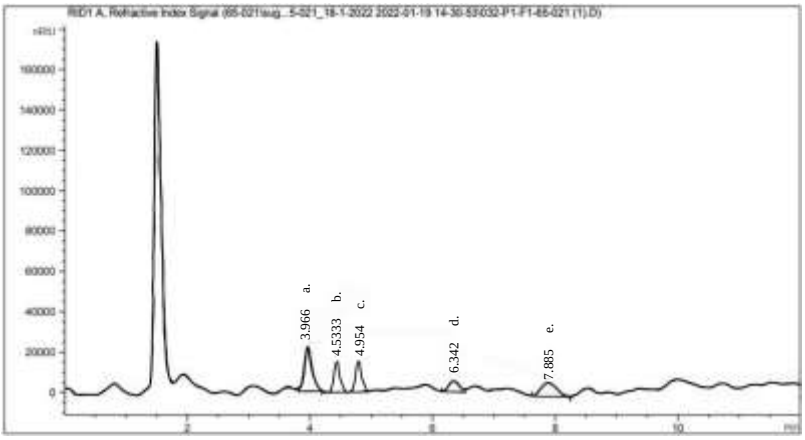


Figure 2 HPLC-RID chromatogram of the standard sugars, fructose (a), glucose (b), galactose (c), sucrose (d) and maltose (e)

The date juice from fresh fruit and processed products i.e. boiled juice, retort juice, and syrup were quantified for those three monosaccharides as well as two disaccharides. All date samples contained only two kinds of monosaccharides, i.e., fructose and glucose, and only one disaccharide, i.e., sucrose, with various amounts [Table 1]. All samples had higher monosaccharides than disaccharides and had a higher amount of glucose than fructose. Apart from that, the retort juice showed a higher amount of fructose than glucose. The total amount of sugar is elevated by the increasing time and temperature of heating. The fresh juice did not proceed through the heat and the fruits were not ripe.

Fresh dates juice showed glucose (46.36%w/v) almost two times higher than fructose (26.46%w/v) and sucrose (27.19%w/v). The boiled juice from ripened date fruit had glucose (47.60%w/v) almost the same ratio as fructose (40.30%w/v) but very less sucrose (12.10%w/v). The syrup presented the same trend as boiled juice, in which glucose (49.19%w/v) was almost the same ratio as fructose (41.92%w/v) but very less sucrose (8.89%w/v). Only retort juice exhibited higher fructose (42.61%w/v) than glucose (32.04%w/v) and sucrose (8.89%w/v).

Table 2 Sugar content in KL-1 date palm fresh juice and processed products

Sample	Amount of sugar in weight (g/100 mL) and percentage					
	Fructose	**Glucose**	**Galactose**	**Sucrose**	**Maltose**	**Total**
Fresh juice	0.1449± 2.52E-05^{d}	0.2539 ± 1.73E-05^{c}	ND	0.1489± 5.80E-06^{b}	ND	0.5477^{d}
	26.46%	46.36%		27.19%		100.00%
Boiled juice	0.2974±1.00E-05^{c}	0.3513±0.00E+00^{b}	ND	0.0893± 4.69E-01^{c}	ND	0.7380^{c}
	40.30%	47.60%		12.10%		100.00%
Retort juice	0.5002±1.00E-05^{b}	0.3761±1.00E-05^{b}	ND	0.2977±1.73E-05^{a}	ND	1.174^{b}
	42.61%	32.04%		25.36%		100.00%
Syrup	1.4227±2.31E-05^{a}	1.6696±5.80E-06^{a}	ND	0.3019±2.52E-05^{a}	ND	3.3942^{a}
	41.92%	49.19%		8.89%		100.00%

The statistical significance was calculated by means ± SD, n=3 between the same kind of sugar in the same column and expressed in the different letters ($p < 0.01$).

4.2 Discussion

The thermal degradation of sugars is a very important reaction in beverage production. It is responsible for the yield of sugars obtainable in date palm manufacturing and the characteristics of the final processed products (Quintas *et al*., 2014). The total amount of sugar in heated samples was elevated by increased times and temperatures [Table 1]. The fresh juice revealed the lowest amount of total sugar. The fruits were not ripe and not developed through the heat condition. When dates are ripening, the starch stored in the fruit gradually degrades into disaccharides and monosaccharides. Therefore, all products from ripened dates, such as boiled juice, retort juice, and syrup had higher amounts of sugar than fresh juice. The syrup that was produced in the longest time of heating showed the highest amount of total sugar. The studies of Woo and coworkers (Wo *et al*., 2009, 2010 & 2015) supported our study of the increasing amount of sugar through heating. When date syrup was slowly stirred on a hot plate until viscous, all fructose, glucose, and sucrose increased. The sucrose in boiled juice decreased noticeably compared to fresh juice. The sucrose might be degraded into fructose and glucose, which also increased. Double heating, using higher temperatures, together with added pressure or longer heating, transformed the starch into sucrose, as well as fructose and glucose, which could be seen in both retort juice and syrup. There is a danger of heating for too long, resulting in the breakdown of fructose and glucose into degraded products such as furfural, 5- methyl furfural, HMF, formic acid, lactic acid, and levulinic acid (Kim & Taub, 1993).

In Table 2, all samples showed a higher amount of glucose than fructose. Only the retort juice showed the opposite result and had a high amount of sucrose. This sample was the only one that performed under pressure. This high pressure, together with a high temperature, might transform starch into sucrose. Then, the degraded glucose from sucrose would probably convert into fructose instead [Kumar *et al*., 2020]. All developed products had better taste than fresh fruits, with no astringency and bitterness. The samples were tasted by more than hundreds of people in a different range of ages (18-65), more than 90% like all products. These products showed high potential to commercialize into the market. The intensive studies of product development such as nutritional tests, stability, pH, heavy ions, and microbial contaminations have to be further investigated.

5. Conclusion

Thai cultivar KL1 date palm was shown to be composed of higher amounts of monosaccharides than disaccharides. The heating and pressure during product development increased the amount of all sugars and destroyed the astringency and bitterness of low-quality date fruits. Our study could promote the possibility of developing low-quality date palm, a source of natural sugars, into new products, however, it is only the first step of product development, which requires further intensive study.

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7. References

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