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Chemical composition and quality of date syrup as affected by pectinase/cellulase enzyme treatment

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Abstract

The date palm tree, which is native to the Mediterranean region and originated in the Arabian Gulf area, is now becoming an important commercial crop in Kuwait. Because of the tremendous efforts of the Public Authority for Agriculture and Fisheries Resources, date palm cultivation has developed quickly in Kuwait during the last decade. These newly planted date fruit trees, as well as tissue culture plants being produced and distributed by KISR, would start bearing fruit in a few years. It may not then be possible to consume all the fresh date fruit locally and, subsequently, newer avenues for turning this surplus fruit into value-added products will become a necessity and a commercially viable venture. Technology was developed on a laboratory scale for the production of date syrup from *tamer* fruits of two commercial varieties, *Birhi* and *Safri*, for further use in food products. Both the varieties were found to be high in total sugar contents (about 88%). Among the various extraction procedures employed for producing date syrup, the use of pectinase/cellulase enzymes gave the highest recovery of total soluble solids (68%) compared with control without these enzymes (35%). The CIE L^* a^* b^* color values for diluted as well as concentrated date syrup of *Birhi* variety were found to be lower than the *Safri* variety, indicating lighter color for the former. The results indicate the possibility of employing pectinase/cellulase enzymes to produce concentrated date syrup from *tamer* fruits for use in food product development.

Keywords: Date fruits; Date syrup; Pectinase; Cellulase; Enzymes; Sugars; Soluble solids

1. Introduction

Dates (*Phoenix dactylifera* L.) have been an important crop in the desert regions of Middle Eastern countries and formed the basis for survival of many ancient nomads. This continues to be true today (Mohammed, Shabana, & Mawlod, 1983). At present, approximately 2000 or more different cultivars of date palm are known to exist all over the world, but only a few important ones have been evaluated for their performance and fruit quality. Rygg (1975) gave a detailed description of the date-growing regions of the world, varieties and general cultural practices.

Many advances have taken place in date palm culture, and fruit is processed, shipped and enjoyed throughout the world. However, in many date-growing areas, the industry is still following many of the time-honored methods of production and has not yet caught up with other modern agricultural industries (Vandercook, 1980). Date fruit is a highly nutritious food product, rich in calories and many vitamins and minerals. Date fruit, being exceptionally rich in potassium and extremely low in sodium, is a desirable food for hypertensive persons who are advised to consume low sodium diets. Increase in date fruit production will, therefore, play an extremely significant role in worldwide improvement of the nutritional status of people, with special reference to calories and important minerals (Yousif, Benjamin, Shefa, & Ali, 1976).

It may be appropriate to predict that date fruit cultivation will outlive (as a renewable agricultural crop) any other revenue resource in this part of the world, mainly because of the highly suitable climate for the growth of this valuable desert tree, hence, it deserves better commercial utilization for value-added food products. Due to tissue culture techniques, the cultivation of date palm has picked up tremendously, which would, in a few years' time, make available a large quantitie of date

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fruit for processing. So far, no effort has been made to produce commercial date syrup using hydrolytic enzymes for use in the food and chemical industry in this region, although a considerable potential already exists. Considering the importance of this crop, this research work was carried out for the standardization of conditions to produce date syrup using enzymic preparations on a laboratory scale for use in food product development, based on which, a suitable pilot scale or semi-commercial scale industry could be built for the production of date syrup-based industrial chemicals like food grade citric acid, medical ethanol and enzyme preparations.

2. Materials and methods

2.1. Raw Materials

Date fruits at the *tamer* stage of maturity for two commercial varieties, i.e. *Birhi* and *Safri*, imported from the Kingdom of Saudi Arabia, were procured from the local market. Both these varieties have medium-soft fruits at the *tamer* stage of maturity, with *Birhi* having smaller-sized fruits and the *Safri* having larger-sized fruits. The two varieties were selected to represent two extremes of fruit sizes among date crops. Detailed description about various stages of maturity in date fruits has been reported earlier (Al-Hooti, Sidhu, & Qabazard, 1997a). These samples were analyzed for moisture, ash, protein, total sugars and pectin contents, according to standard AOAC methods (AOAC, 2000). Pectinase and cellulase enzymes were provided free of change by the Enzyme Development Corporation, New York, USA.

2.2. Extraction of date syrup

The *tamer* date fruits of these two varieties were pitted manually. Weight of pulp and seeds were recorded. Based on a number of preliminary trials, date pulp:water ratios, extraction temperature–time combinations, pH and centrifugation conditions were standardized over a wider range. Later, only a few of the optimized extraction conditions were further studied. The date pulp was homogenized with varying pulp:water ratios (1:2, 1:3, 1:4) using a hand-held blender (Phillips, Holland). Each sample, in duplicate, was placed in a water bath at 80 °C for 1, 2 and 3 h. They were then centrifuged (model RC28S, Sorvall, USA) at 10,816 ***g*** for 25 min, and the supernatant was decanted and weighed.

The extraction procedure was repeated using the mentioned pulp:water ratios, but at 100 °C for 10, 15 and 20 min. After centrifuging at 10,816 ***g*** for 25 min, the supernatant was decanted and weighed. The extraction procedure was also repeated with an autoclaving step using 15 psi steam pressure for 5, 10 and 15 min, and the supernatant decanted and weighed. These extraction procedures were repeated, but after the pH of pulp:water mixture was adjusted between 5.5 and 6.5 using 0.1 N hydrochloric acid or 0.1 N sodium hydroxide.

The date fruit pulp was blended with three times the water and pH was adjusted to 6.0 ± 0.2 before the addition of enzyme preparations. Pectinase enzyme preparation was obtained in already available liquid form, but, in the case of cellulase, 250 mg of enzyme powder was dissolved in 100 ml water before use. Pectinase and cellulase enzyme preparations were added at the rate of 0.5, 1.0 and 2.0% on a pulp basis. The pulp and enzyme mixture was incubated at 40 °C for varying periods up to 24 h. At the end of the incubation period, it was centrifuged and the supernatant collected to compute the yield of total soluble solids. To test the effect of pectinase and cellulase enzyme preparations on the viscosity, a drop in apparent viscosity of this pulp:water slurry was measured at 1 h intervals for 5 h as well as after overnight (i.e. 16 h) using a Brookfield viscosimeter (model LVDV-II, Brookfield Eng. Lab., USA). The viscosity was measured at 25 ± 1 °C using spindle no. 2, RPM of 60 and in a 250-ml capacity glass beaker (60 mm diameter). The pulp:water slurry was then centrifuged at 10,816 ***g*** for 25 min, and the supernatant decanted and weighed. The recovery of soluble solids (RSS) was computed as follows:

$$\mathrm{RSS} = \frac{\text{Weight of extract} \times \%\ \text{TSS of extract}}{\text{Weight of date pulp taken}} \times 100$$

2.3. Chemical analysis of concentrated date syrup

The date syrup prepared by all the extraction treatments was pooled for *Birhi* and *Safri* varieties and vacuum concentrated at 60 °C using a rotary evaporator (model Rotavapor R-124, Buchi, Switzerland) to 80.4 and 81.2°Brix, respectively. The concentrated date syrup samples were analyzed for pH, acidity, ash, protein using standard AOAC methods (AOAC, 2000). These samples were also analyzed for glucose, fructose (using HPLC), a few of the macro- and micro-elements (inductively coupled plasma technique using atomic emission spectrophotometer) according to the methods described earlier (Al-Hooti, Sidhu, & Qabazard, 1997b).

2.4. *CIE* L*a*b* color measurement of date syrup

The CIE $L^*a^*b^*$ color values for date syrup prepared by autoclaving, enzyme treatment methods and the concentrated syrup, were measured with a Mcbeth Color Checker as per the procedure described by Al-Hooti et al. (1997a, 1997b). Under this tristimulus color

coordinate system, the L^* value is a measure of lightness and varies from 0 (black) to 100 (white); the a^* value varies from −100 (green) to +100 (red); and the b^* value varies from −100 (blue) to +100 (yellow). As the values of a^* and b^* rise, the color becomes more saturated or chromatic, but these values approach zero for neutral colors (white, grey or black).

2.5. Statistical analysis

Most of the chemical analyses are reported on a moisture-free basis. The experimental data obtained were analyzed statistically for analysis of variance, and mean values were evaluated for statistical significance ($P = 0.05$) using Duncan's new multiple range test (SAS Program Windows Version 6.08) and the inferences reported in appropriate places. Significance was accepted at the $P = 0.05$ level. For other results, the mean values and standard deviations are reported.

3. Results and discussion

3.1. Proximate analysis

The date fruits from *Birhi* and *Safri* varieties were analyzed for moisture, ash, protein, total sugars and pectin, and the results are presented in Table 1. The date fruit pulp from *Birhi* and *Safri* varieties had a moisture content of 11.55 and 11.53%, respectively. The ash, protein, total sugars (as reducing sugars) and pectin contents were found to be 2.08 and 2.16, 2.60 and 2.03, 88.02 and 87.53, 0.56 and 0.44% for *Birhi* and *Safri* varieties, respectively. The fat content was less than 0.1% and was not reported. The pulp:seed ratio for *Birhi* and *Safri* varieties was found to be 84:16 and 88.5:11.5, respectively.

3.2. Extraction conditions for soluble solids

The date pulp was taken for the extraction of soluble solids using varying ratios of pulp:water and temperature conditions. After the preliminary trials, a few of the pulp:water ratios and temperature–time combinations were selected for further studies. The effect of varying ratios of pulp:water, the extraction times and temperatures on the yield of soluble solids are presented in Tables 2–4. Although the pulp from variety *Birhi* gave slightly higher yields of soluble solids than the *Safri* variety under all the extraction conditions, the differences were statistically insignificant. The yield of soluble solids from these varieties ranged from 30.17 to 35.09%, with varying ratios of pulp:water as well as temperature–time combinations. These yields of soluble solids based on a pulp basis are considered to be extremely low and cannot be economically feasible to scale-up to commercial levels. Considering this, attempts were made to increase the yield of soluble solids to a reasonable level using pectinase/cellulase enzyme preparations.

3.3. Enzyme treatments and yield of soluble solids

Pectolytic enzymes have been used for increasing the yield of juice from stone fruits like peaches, plums and apricots (Joshi, Chauham, & Lal, 1991). No such attempt to use pectinase enzymes in the extraction of soluble solids from date fruits has been reported so far. By using these enzyme preparations, the extraction yield

Table 1
Chemical composition of *tamer* date fruits of different varieties

Variety	Moisture content (%)	Ash[a] (%)	Protein[a] (N × 6.25;%)	Total sugars[a] (as reducing sugars;%)	Total pectin[a] (as Ca pectate;%)
Birhi	11.55±0.19	2.08±0.10	2.60±0.07	88.02±1.06	0.56±.02
Safri	11.53±0.10	2.16±0.14	2.03±0.11	87.53±1.15	0.44±0.02

[a] On moisture-free basis

Table 2
Effect of extraction temperature (80 °C), time and varying pulp:water ratios on the yield of soluble solids (%) from *tamer* date fruits of different varieties

Variety	Extraction time (h)								
	1			2			3		
	Date: water ratio								
	1:2	1:3	1:4	1:2	1:3	1:4	1:2	1:3	1:4
Birhi	33.15±0.77	33.09±0.63	33.91±0.50	33.10±0.74	34.32±0.51	33.71±0.50	32.18±0.88	34.13±0.69	34.67±0.49
Safri	32.47±0.67	33.48±0.52	32.56±0.60	31.83±0.57	32.66±0.85	32.84±0.33	32.48±0.47	32.68±0.42	33.02±0.93

Table 3
Effect of extraction temperature (100 °C), time and varying pulp:water ratios on the yield of soluble solids (%) from *tamer* date fruits of different varieties

Variety	Extraction time (min)								
	10			15			20		
	Date:water ratio								
	1:2	1:3	1:4	1:2	1:3	1:4	1:2	1:3	1:4
Birhi	33.07±0.54	34.31±0.35	33.97±0.70	33.33±0.94	34.98±0.91	35.20±0.40	33.70±0.41	34.94±0.61	35.09±0.37
Safri	31.24±0.49	32.01±0.94	31.85±0.45	30.17±0.74	31.53±0.84	31.43±0.47	31.31±0.43	32.30±0.61	32.12±0.81

Table 4
Effect of autoclaving conditions (15 psi), time and varying pulp:water ratios on the yield of soluble solids (%) from *tamer* date fruits of different varieties

Variety	Autoclaving time (min)								
	5			10			15		
	Date:water ratio								
	1:2	1:3	1:4	1:2	1:3	1:4	1:2	1:3	1:4
Birhi	31.60±0.60	32.55±0.71	32.53±0.73	31.27±0.81	32.57±0.73	33.14±0.69	32.85±0.37	33.27±0.93	33.85±0.68
Safri	31.44±0.48	32.03±0.88	32.37±0.86	31.36±0.50	32.11±0.60	32.19±0.81	30.89±0.74	32.11±0.74	32.93±0.85

of soluble solids was enhanced to 67.5 and 68.22% for *Birhi* and *Safri* varieties, respectively. The extraction yields almost doubled with the enzyme preparations compared with that of hot water or autoclaving techniques. The addition of enzyme preparations, even at the level of 0.5%, was found to increase the extraction yield of soluble solids from about 63.10 to 66.58% for these varieties (Table 5). The use of 2.0% enzyme level was not significantly different than the 1% level, so the use of 1% pectinase and cellulase enzyme preparations was found to be optimal for the maximum extraction yields of soluble solids from date fruit pulps. As the extraction yields of soluble solids from date pulps increased by almost 100%, this may open up the avenue for the use of such enzyme preparations for date fruit pulp processing for the development of sucrose substitutes based on date fruits.

Table 5
Effect of pectinase and cellulase enzyme preparations on the extraction yield (%) of soluble solids from *tamer* date fruits of different varietie

Variety	Concentration (ml/100g) of pectinase and cellulase enzyme preparations used		
	0.5	1.0	2.0
Birhi	63.10±1.02	67.50±0.75	66.09±0.84
Safri	66.58±1.17	68.22±0.76	67.97±0.85

3.4. Enzyme treatment and viscosity of date fruit pulp

The apparent viscosity of this mixture was measured at hourly intervals up to 5 h and then after 24 h, using a Brookfield viscosimeter, and the results are presented in Table 6. As can be seen from this data, the apparent viscosity of date fruit pulp increased at 1 h and then consistently decreased with the passage of time at all enzyme concentrations used in this study. The *Birhi* variety gave slightly higher apparent viscosity than the *Safri* variety; probably, the former had a higher pectin content (Table 1). There was almost 100% reduction in the viscosity values among all the pulp samples treated with pectinase/cellulase enzyme preparations. The reduction in the viscosity of other fruit juices due to enzymatic hydrolysis of pectin has been reported by a number of workers (Gupta & Girish, 1988; Urlaub, 1996). The initial slight increase in viscosity of date fruit pulp may possibly be due to the increased extraction of pectic substances through the action of these enzymes on the cell wall materials of date fruits.

3.5. Extraction treatments and date syrup CIE L* a* b* color values

The CIE *L*a*b** color values for date syrup prepared by autoclaving as well as enzyme treatment methods were measured with a Mcbeth ColorChecker as per the procedure described by Al-Hooti et al. (1997a, 1997b),

Table 6
Effect of dosage of pectinase and cellulase enzymes (ml/100 g) and the incubation time (h) on the apparent viscosity (cP) of date fruit pulp

Time of incubation (h)	*Birhi*			Safri		
	Pectinase and cellulase enzyme preparation used (ml/100 g)					
	0.5	1.0	2.0	0.5	1.0	2.0
0	24.2±2.97	29.3±1.49	29.7±1.27	20.3±1.27	23.5±1.27	23.4±3.18
1	34.9±1.13	42.4±0.92	32.9±1.06	22.7±3.96	25.9±1.34	24.1±3.32
2	30.1±0.70	30.8±0.50	27.0±1.41	20.7±1.56	23.2±4.59	17.4±4.38
3	25.7±1.06	24.7±0.50	19.5±0.14	17.0±4.17	18.9±3.75	16.8±4.45
4	24.1±1.41	21.3±2.97	17.5±0.35	14.5±2.69	15.6±4.03	13.3±1.91
5	22.0±0.64	17.6±2.12	16.0±1.70	13.0±1.06	14.8±3.67	12.8±1.00
24	14.8±0.64	12.8±0.50	12.5±0.84	11.5±2.40	12.1±0.85	10.2±0.92

Table 7
CIE L^* a^* b^* color values of date syrups prepared from different varieties using pectinase/cellulase enzymes and autoclaving treatments

Variety	Pectinase and cellulase enzymes			Autoclaving		
	L^*	a^*	b^*	L^*	a^*	b^*
Birhi	10.93±0.91a	3.80±0.36g	5.30±0.79p	7.02±0.88b	0.29±0.16h	0.77±0.16q
Safri	8.05±0.52c	1.79±0.25d	1.76±0.38r	4.53±0.37d	1.68±0.17d	0.69±.26q

L^* value is a measure of lightness ranging from 0 (black) to 100 (white), the a^* value ranges from −100 (greenness) to +100 (redness), and the b^* value ranges from −100 (blueness) to +100 (yellowness). For judging significance in the CIE L^* a^* b^* color values of date syrups, the letters should be compared for different varieties within a column and/or in a row between the enzyme and autoclaving treatments ($P<0.01$).

and the results are presented in Table 7. The CIE $L^*a^*b^*$ color values were found to be affected significantly by different treatments as well as by the variety. The diluted syrup was found to possess lighter color (higher Lightness value) for both the treatments for the *Birhi* variety than the *Safri*. The autoclaving treatment resulted in darker color for both the varieties (a^* b^* values approaching zero) compared with the enzyme-treated syrup. The higher a^* b^* values (indicating color intensity or hue) for the enzyme-treated date syrup prepared from *Birhi* variety indicate their tinge to be on the red/yellow side compared with the enzyme-treated syrup from the *Safri* variety. Autoclaving treatment was reported earlier to darken the visual color of syrup from other date fruit varieties (El-Shaarawy, Messalam, El-Nakahal, & Wahdan, 1986). Comparing these results, enzyme treatment holds great promise in obtaining better quality date syrup for further use in food products.

The CIE $L^*a^*b^*$ color values of pooled concentrated date syrup (about 80°Brix) were also measured by this procedure and the results are presented in Table 8. The concentrated syrup from the *Birhi* variety possessed a significantly lighter color (higher Lightness value) than the *Safri* variety. As the a^* b^* values (indicating color intensity or hue) approached zero, it indicated that the color of these concentrated syrup samples attained a darker red/yellow color, in spite of the fact that these syrup samples were concentrated under vacuum in a rotary evaporator at 60 °C. Visually, the concentrated syrup samples had the appearance of a natural honey as far as their color, appearance, taste and viscosity were concerned. As the date syrup had color and flavor similar to honey, it was used in the preparation of a few baked products, the results of that study are being reported

Table 8
CIE L^* a^* b^* color values of pooled concentrated date syrups prepared from different varieties using pectinase/cellulase enzymes and heating treatments

Variety	CIE L^* a^* b^* objective color values		
	L^*	a^*	b^*
Birhi	8.57±0.83a	0.72±0.15c	2.76±0.28e
Safri	5.94±0.19b	0.99±0.10d	2.15±0.19f

L^* value is a measure of lightness ranging from 0 (black) to 100 (white), the a^* value ranges from −100 (greenness) to +100 (redness), and the b^* value ranges from −100 (blueness) to +100 (yellowness). For judging significance in the CIE L^* a^* b^* color values of concentrated date syrups, the superscripts should be compared for different varieties within a column only ($P<0.01$).

Table 9
Physicochemical composition (as is basis) of concentrated date syrup prepared from *Birhi* and *Safri* varieties

Variety	Moisture content %	pH	Acidity %, (as citric acid)	Glucose %	Fructose %	Ash %	Protein %
Birhi	16.76	4.09	0.77	38.02	39.12	1.77	1.68
Safri	16.25	4.11	0.67	38.45	39.69	1.51	1.22

Table 10
Mineral composition of concentrated date syrup prepared from *Birhi* and *Safri* varieties (mg/100 g, dry basis)

Variety	Macro-elements					Micro-elements			
	Ca	P	Na	K	Mg	Fe	Zn	Cu	Mn
Birhi	35.4	152.3	673.0	531.8	28.4	0.10	0.31	0.13	0.20
Safri	26.0	138.1	595.0	497.9	22.7	0.10	0.28	0.18	0.29

separately. The apparent viscosity of concentrated syrup of *Birhi* and *Safri* was measured in the same way as explained earlier, except that spindle no. 3 and RPM of 10 were used. The apparent viscosity values for the concentrated syrup from *Birhi* and *Safri* varieties were found to be 389 and 446 cP, respectively. The physico-chemical composition and mineral contents of concentrated date syrup have been presented in Tables 9 and 10, respectively.

4. Conclusions

The research data presented here indicate a strong possibility of producing concentrated date syrup for use in product development. Both the varieties were found to be high in total sugar contents (about 88%). Among the various extraction procedures employed for producing date syrup, the use of pectinase/cellulase enzymes gave the highest recovery of total soluble solids (68%) compared with the control without these enzymes (35%). Glucose and fructose were the major sugars present in date syrup. The date syrup was also found to be a good source of macro-elements like calcium, phosphorus, potassium, magnesium, but significantly low in sodium. The CIE L^* a^* b^* color values for diluted as well as concentrated date syrup of *Birhi* variety were found to be lower than the *Safri* variety, indicating lighter color for the former. It can be concluded that there is good potential and a possibility to produce syrup from date fruits for use as a replacement of sucrose in food products. Further studies are suggested for the use of these enzymes for the production of syrup from date fruits at other stages of maturity viz. *kimri, khalal* and *rutab*.

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