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Utilization of enzymes in the production of liquid sugar from dates

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Date palm fruits *Phoenix dactylifera* cultivated in the Kingdom of Saudi Arabia were used for the production of high quality date syrup. This syrup is suitable for the manufacture of different food products. Date syrup was prepared from Reziz date (Soft variety) at different ratios of water/date (2:1, 2.5:1 and 3:1) . Pectinase and cellulase were used to obtain the maximum date syrup extraction. Data revealed that Reziz variety contained high total sugar content (about 83.51% on dry basis). The extraction rate of sugars increased as the water/flesh ratio increased. Also, the use of pectinase/cellulase gave the highest recovery of total soluble solids (65.6 to 70.7%) compared with control (50.5 to 56.30%). The resultant date syrups were evaluated for their physico-chemical characteristics and compared with cane syrup (Black honey). Results of the organoleptic evaluation proved that date syrup is considered to be highly desirable than cane syrup. Results indicate the possibility of employing pectinase/cellulase to produce concentrated date syrup from tamr fruits for use in food product development.

Key words: Date fruits, Reziz, date syrup, pectinase, cellulose, physico-chemical characteristics.

INTRODUCTION

The date palm *Phoenix dactylifera* is one of the oldest fruit trees in the Arabian Peninsula and has played an important role in the day-to-day life of the people of this region for the last 7000 years (Ahmed et al., 1995). Dates are known to be rich in carbohydrates (80%) but quite low in protein (2 - 3%) (Al-Hooti et al., 1997). Dates are an excellent source of simple sugars, minerals and vitamins (El-Shaarawy et al., 1989) and its fiber content reaches about 8% (FAO, 2004; Lambiote, 1982). The flesh of a fully ripe date (Tamr), consist of two- third sugars and one-quarter water, the rest being mainly cellulose, pectin, ash and vitamins (FAO, 1992). The date is considered as a nutritious fruit as research has indicated the clear contribution of dates to human health when consumed with other food constituents (Lambiote, 1982). Dates contain sufficient quantities of minerals and vitamins that help to prevent deficiencies. Dates also have an sugar. Date fruits (Tamr) contains moisture ranging from 10 to 22%, total sugars 62 to 75%, protein 2.2 to 2.7%, fiber 5 to 8%, fat 0.4 to 0.7%, ash 3.5 to 4.2%, total acidity 0.06 to 0.20% and ascorbic acid 30.0 to 50.0 mg%, on dry weight basis (FAO, 1992; Baraem et al., 2006; El-Sharnouby et al., 2007).

The production of date fruits in the world reached about 6.7 million tonnes (FAO, 2004). The development of date fruits is divided into three stages, Khalal, Rutab and Tamr. Khalal stage dates are immature with hard texture, yellow, red or pink in colour, total soluble solids (TSS) of 30 – 45 °brix, astringent and edible; Rutab stage dates soften at the tip of the fruit, with TSS of 55 - 60 Brix, free from astringency and edible; Tamr stage dates are fully riped with TSS of 60 - 84° Brix and edible (Pareek, 1985). Dates are generally harvested at the Tamr stage that is after the development of TSS of 60 – 70 °brix that are edible at this stage. Date syrup are used in food preparations like sweets, snacks, confectionery, bakery products and health foods (Riedel, 1986). Mature date fruits are also processed into products such as date bars, date syrup, etc. (Abd El-Mohsen and Nezam El- Din, 1995). Date juice extraction and date syrup were studied by 1983), El-Shaarawy et al. (1989),

Mustafa et al. (1989), Ramadan (1995) and Al-Hooti et al. (2002). Date syrup (dibs) is probably the most com-

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mom derived date product. It is produced as an incidental by-product when bagged humid dates are heaped for several months, some syrup oozes out by the force of their own weight. Also, it is produced in the home and village by extraction and boiling down of juice and on a semi and full industrial scale (FAO, 1992).

Most dates are consumed at the Rutab (semi-ripe) and Tamr (fully-ripe) stages, with little or no processing. The quantities of processed dates have been rapidly and steadily growing in recent years because of encouragement and support provided to the date processing Industry by the government (Al-Hooti et al., 1997). Today, worldwide production, utilization and industrialization of dates are continuously increasing (FAO, 2004). In the Kingdom of Saudi Arabia, dates are one of the most important crops because of their religious and nutritional significance (El-Behissy et al., 1998). Dates production in Saudi Arabia greatly increased over the past two decades, and the latest Ministry of Agriculture figures estimate it at 970,488 tonnes (Ministry of Agriculture, 2006). This increase is also paralleled by high consumption. The per capita daily consumption of dates in Saudi Arabia was estimated to be between 104 gm (Ministry of Agriculture, 2006).

Many studies have also been carried out on date by-products such as date paste, pickles and jams. In food technology, date syrup as the main and general by-product of date is used for foodstuffs such as jams, marmalades, concentrated beverages, chocolates, ice cream, confectioneries and honey e.t.c. As the Kabkab date has a high amount of wastes, it can be used for date syrup production with economical advantages (Al- Hooti et al., 1997; Hamad et al., 1983; Khatchadourain et al., 1983).

In date syrup industry, the fruit are mixed with water and heated for around 1 h at 50°C and the main compo-nent, sugars, are then extracted. This method destroys some nutritive components and darkens the product's color. Therefore, this work was carried out for the utilization of enzymes (pectinase and cellulase) and filtration system in the production of liquid sugar from dates for use in food product development.

MATERIALS AND METHODS

Raw materials

Date fruits (*P. dactylifera* L.) at the Tamr stage of maturity (soft variety) for one date variety, that is, Reziz, which is considered a good quality obtained from Al- Hassa Govemorate, Date Factory, King-dom of Saudi Arabia was used in this work. Representative part of the date bulk was used for some physical evaluation. Another part was pitted and the flesh was minced just before chemical analysis. The residue fruits were washed and pitted while the flesh was cut into small pieces and kept refrigerated in sealed polyethylene bags.

Pectinase and cellulase (in liquid state) were provided from the Sigma Company (New York, USA). Pectinase (9,500 units/ml) and cellulase (1000 units/g) were mixed at a ratio 1:1 and added at 0.25, 0.50 and 1.0% on date pulp.

Chemicals

All chemicals and solvents were obtained from the Sigma-Aldrich Co. (Dorset, UK), unless otherwise specified.

Extraction of the date syrup

The water/date pulp ratios (W/D) were (1:1, 2.5:1, 3:1) without the addition of enzymes (as control samples). The extraction procedure was also repeated but with the addition of pectinase and cellulase preparations (1:1) at the rate of 0.5, 0.75 and 1.0 % (v/w) on a pulp weight basis for each mentioned above pulp: water ratio and pH was adjusted to 6.0 ± 0.2 before the addition of the enzyme preparations. Each sample, in duplicate, was placed in an incubator at 40°C for 24 h. After incubation, samples were blended using a hand-held blender (Phillips, Holland) and then the slurry was filtered through a cheese cloth with a hand press. The obtained extract was filtered in a filtration system (Novox 200, filtrox, Swiss) for fine purification. The clear extract was concentrated to about 72 °brix using a rotary evaporator (Rotavapor R-124, Buchi, Switzerland) at 70°C (Al- Hooti et al., 2002) . The concentrated samples were packed in sealed glass bottles and stored at room temperature (20 - 30°C) until analyzed and evaluated. However, the recovery of soluble solids (RSS %) was computed as follows:

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

Physical and chemical analyses

The date fruit samples were evaluated for number of fruits per kg, weights of fruit, flesh and pit, flesh/pit ratio and fruit flesh percentage. These samples were analyzed for moisture, ash, protein, total sugars and pectin contents, according to standard methods (AOAC, 2000) . Total soluble solids (°brix) were determined by means of the Abbe refractometer (Milton Roy, USA). The color of the diluted date syrup (20% TSS) was measured as optical density at 520 nm using a spectrophotometer (Cecil 144-104, England). The extracted date syrup from each treatment was pooled and vacuum concentrated at 60°C using a rotary evaporator (Rotavapor R-124, Buchi, Switzerland) to about 72 °brix. The concentrated date syrup samples were analyzed for pH, acidity, ash, protein using standard methods (AOAC, 2000). These samples were also analyzed for glucose and fructose (using HPLC). Potassium and sodium were determined using a Flame photometer. Calcium, magnesium and iron were determined using the Atomic absorption spectrophotometer 2380 as described in AOAC (2000).

Organoleptic evaluation

Organoleptic evaluation of date syrup samples were determined by a taste panel comprising of 15 staff members at King Faisal University, Saudi Arabia. The panel scoring system applied was: color (20 points), taste (30 points), consistency (20 points) and acceptability (30 points). This system was applied for some date products by Sumainah and EI-Nakhal (1984), Ramadan (1995) and Yousif et al. (1989) . Data were subjected to analysis of variance and least significant difference (LSD) at 5% probability according to Snedecor and Cochran (1980). However, evaluated date syrup was compared with the sugar cane syrup (black honey) from the local market (Al-Hassa, Saudi Arabia).

Statistical analysis

The obtained experimental data were statistically subjected to

Table 1. Physical characteristics of Reziz date fruits.

Date sample	Fruit (no./kg)	Fruit weight (g)	Flesh weight (g)	Pit weight (g)	Pit/ Flesh ratio	Fruit flesh %
Reziz	175	6.67	5.67	1.00	17.63	85.00

Table 2. Chemical composition* of date fruits (on dry basis).

Date sample	Moisture %	Total sugars (as reducing sugar, %)	Crude protein (%)	Ash (%)	Total Pectin as Ca pectate (%)
Reziz	11.50	83.51	2.21	2.01	0.50

*Average of three replicates.

Table 3. Effect of water/date pulp ratio (W/D) on the extraction yield (RSS %) of soluble solids from Reziz date fruit.

Water/date pulp (W/D)	2/1	2.5/1	3/1
RSS	50.50	52.20	56.30

analysis of variance and least significant difference (LSD) at 5% probability according to Snedecor and Cochran (1980).

RESULTS AND DISCUSSION

Physical and chemical evaluation of Reziz date

Data presented in Table 1 showed that, the number of fruits/kg was 175 fruits for Reziz date. Consequently, the mean weights of fruit and flesh of Reziz date were 6.67 and 5.67 g. It could be noted also from Table 1 that, Reziz date had a pit /flesh ratio of 17.63 and fruits flesh percentage of 85%. These results are in line with that reported by Nour et al. (1989), Fayadh and Al-Showiman (1990) and Ramadan (1995).

The data of gross chemical composition are shown in Table 2. It could be concluded that date samples contained predominant amount of sugars and moderate levels of crude protein and ash. Reziz date variety recorded a high amount of sugars (83.51% on dry basis) and crude protein (2.21%). The obtained data are in close agreement or even less than that reported by Yousif et al. (1982), Nour et al. (1989), Ramadan (1995) and El-Sharnouby et al. (2007).

Al-Hooti et al. (1997) and Al-Farsi et al. (2007) found that Tamr dates had a moisture content less than 15%. However, it was clear from the data in Table 2 that, the moisture content of dates was less than the recorded average of semi-dry varieties (15%).

Extraction of Reziz date juice

The effect of water to date pulp (W/D) ratio on sugar extraction rate (RSS %) and physical characteristics of date syrup of the extracted date juice is presented in Tables 3 and 4. These data revealed that, there was a positive relationship between the extraction rate of sugar and W/D ratio. This positive relationship could be due to the increased rate of molecular diffusion, reduced liquid viscosity and better liquid solid contact. Consequently, the increase in the extraction rate caused the residual sugar in the draft date to be decreased. The results obtained are in agreement with the results of Ramadan (1995).

Moreover, it is evident from data in Table 3 that, RSS % was relatively affected by the-moisture content of date pulp. Whereas, date fruits (at ratio 3:1 W/D) recorded the highest RSS % (56.30%). Benjamin et al. (1982) reported that water to date ratio was evidently a very important parameter in solids extraction for technical and economic reasons. A high ratio will assist rapid and thorough extraction. However, the low TSS of the extract is not easy to preserve on an industrial scale and will require very high energy to concentrate. However the results obtained are in agreement with the results of Al-Hooti et al. (2002) and Al-Farsi et al. (2007) . Moreover, the best RSS % was obtained with a ratio of 3:1 water/date pulp, thus this ratio was chosen in further experiments.

Date syrup evaluation

Data concerning the physical characteristics of date syrup is presented in Table 4. The total soluble solids (TSS %) and refractive index of the date syrup were 75.50% and 1.4776, respectively. This is due to the high level of sugars and relatively low moisture content. The pH value recorded was 4.12 for date syrups. The high level of acidity in syrups contributed to its stability against micro-

Table 4. Characteristics of date syrup (at ratio 3/1 water/date pulp).

Date syrup	Syrup/date pulp (g/Kg)	TSS (%)	Refractive index (at 20°C)	Acidity % (as citric acid)	pH	O.D. of 20% TSS (at 520 nm)
Reziz syrup	890	75.50	1.4776	0.66	4.90	0.51

Table 5. Chemical composition of date syrup.

Samples	Moisture (%)	Total sugars (as reducing sugar, %)	Crude protein (%)	Ash (%)	Total Pectin as Ca pectate (%)
Reziz fruits	11.50	83.51	2.21	2.01	0.50
Reziz syrup*	18.80	74.70	1.46	1.62	0.00

Average of three replicates; *at ratio 3/1 water/date pulp without enzymes.

Table 6. Mineral composition of date syrup at ratio 3/1 water/date pulp, (mg/100 g,dry basis).

Date syrup	Macro elements					Micro elements			
	Ca	P	Na	K	Mg	Fe	Zn	Cu	Mn
Reziz	71.50	160.20	620.20	940.30	45.20	0.80	0.50	0.60	0.25

Table 7. Effect of pectinase/cellulose enzyme preparations (ml/100 g) on the extraction yield (RSS %) at ratio 3/1 water/date pulp of soluble solids from Reziz date.

Extraction yield RSS %	Water/date pulp (W/D) without enzyme		
	2/1	2.5/1	3/1
RSS*	50.50	52.20	56.30
	Concentration (ml/ 100g) of pectinase and cellulose enzymes		
	0.25	0.50	1.0
RSS**	70.30	74.40	78.60

*Without enzymes; ** at ratio 3/1 water/date pulp.

organisms. The optical density of 20% TSS of date syrups at 520 nm was 0.51. The results confirm those previously recorded by Ramadan (1995). The data (Table 4) showed that the prepared date syrup had a high acidity of 0.66% (as citric acid) and total solids contents of 75.50 %, therefore the expected storage ability will be high. Moreover, it was concluded from the data in Table 4 that 1 kg of each date pulp could produce about 890 g of syrup with 18.80% moisture content.

The chemical compositions of date syrup are mentioned in Table 5. It was observed that the moisture content of date syrup was 18.80%. The reducing sugars of date syrup was 74.70%, crude protein 1.46% and ash content 1.62%. Pectin content was not detected in date syrup. This is due to the impact of using a filter system (Novox 200, filtrox) which could result entirely in a clear juice. The obtained data are in line with that reported by Al-Hooti et al. (2002), Entezari et al. (2004) and Al-Farsi et al. (2007).

The mineral contents of concentrated date are presented in Table 6. Date syrup is a good source of elements such as calcium, phosphorus, potassium, iron, zinc and cupper. However, the obtained results are in accordance with the results obtained by Mohamed (1999), Al-Hooti et al. (2002) and Baraem et al. (2006).

Effect of pectinase and cellulase enzymes

Effect of pectinase and cellulose preparations on the recovery of soluble solids (RSS %) at ratio 3:1 water: date pulp from Reziz date is shown in Table 7. Three treatments of pectinase and cellulase preparations, 0.25, 0.50 and 1.0 ml/100g pulp date were used. Pectolytic enzymes have been used for increasing the yield of juice

Table 8. Effect of pectinase/cellulase (1.0 ml/100 g) (at ratio 1:3) on chemical composition of date syrup

Samples	Moisture %	Total sugars (as reducing sugar, %)	Crude protein (%)	Ash (%)	Total Pectin as Ca pectate(%)
Reziz fruits	11.50	83.51	2.21	2.01	0.50
Reziz syrup*	18.80	74.70	1.46	1.62	0.00
Reziz syrup**	15.50	79.60	1.58	1.80	0.00

*At ratio 3/1 water/date pulp without enzymes; **at ratio 3/1 water/date pulp with pectinase/cellulase (1.0 ml/100 g).

Table 9. Mineral composition of concentrated date syrup prepared from Reziz pulp date with pectinase/cellulase (at ratio 3/1water/date pulp, g/100g, dry basis) as Ca pectate(%).

Date	Macro elements					Micro elements			
Syrup	Ca	P	Na	K	Mg	Fe	Zn	Cu	Mn
Reziz ruits	71.50	160.20	620.20	940.30	45.20	0.80	0.50	0.60	0.25
1*	66.30	168.21	630.51	989.21	52.13	1.00	0.66	0.72	0.32
2**	71.50	160.20	650.20	940.30	45.20	0.80	0.50	0.60	0.25

*Date Syrup without enzymes ** Date Syrup with enzymes 1.0%.

Table 10. Physicochemical composition of concentrated date syrup prepared from Reziz date pulp (at ratio 3/1water/date pulp).

Date Syrup	pH	Acidity% (ascitric cid)	Glucose(%	Fructose(%)
1 *	4.12	0.66	37.50	37.20
2 **	4.08	0.75	40.45	39.15

*Date syrup without enzymes; ** date Syrup with enzymes 1.0%

from stone fruits like peaches, plums and apricots. No such attempt to use pectinase enzymes in the extraction of soluble solids from date fruits has been reported so far (Al-Hooti et al., 2002). By using these enzyme preparations, the extraction yield of soluble solids for Reziz date variety was enhanced. The addition of enzyme preparations, even at the level of 1.0%, was found to increase the extraction yield of soluble solids from 73.30 to 78.70% (Table 7). As the extraction yields of soluble solids from date pulps increased, this may open up the avenue for the use of such enzyme preparations for date fruit pulp processing for the development of sucrose sub-stitutes based on Date fruits. However, the use of 2.0% enzyme level was not significantly different than the 1% level and so the use of 1% pectinase and cellulase preparations was found to be optimal for the maximum extraction yields of soluble solids from date fruit pulps (Al-Hooti et al., 2002).

The effect of pectinase and cellulase preparations at 1.0 ml/100 g pulp date on the chemical composition of date syrup (at ratio 3:1 of extraction, on a dry basis) is shown in Table 8. It was observed that the moisture content in date syrup was 18.80% and became 15.50% on addition of pectinase and cellulose preparations. Data obtained also showed that, the reducing sugars of date syrups was 74.70% and increased to 79.60% with the enzyme mixture. Also, crude protein and ash contents slightly increased. Moreover, pectin content was not detected in date syrup. This was due to the effect of the enzyme mixture on date cells because more extraction of total soluble solids takes place and also the use of a filtration system (Novox 200, filtrox,) through which entirely clear juice could be obtained. The present data are in line with that reported by Entezari et al. (2004) and Al-Farsi et al. (2007).

Mineral composition of concentrated date syrup prepared from Reziz pulp date with pectinase and cellulase (at ratio 3:1 water: date pulp, mg/100 g, dry basis) are tabulated in Table 9. Date syrup is a good source of elements such as calcium, phosphorus, potassium, iron, zinc and copper. The present data are in line with that reported by Mikki et al. (1983) and Al-Hooti et al. (2002). It could be concluded from the obtained results that the highest amounts of minerals and phosphorus were found after the addition of the enzymes mixture. Results obtained from the analyses of data are in accordance with the results obtained by Mohamed (1999) and Baraem et al. (2006).

The physico-chemical compositions of concentrated date syrup before and after the addition of pectinnase/cellulase have been presented in Table 10. Slight increases in acidity and reducing sugars and no changes

Table 11. Analysis of variance for organoleptic evaluation of date syrup at ratio 3:1 with pectinase and cellulase compared with control sample and black honey.

Syrup	Color (20)	Taste (30)	Consistency 20)	Acceptability (30)	Total score(100)
Control*	16.20ab	26.50a	17.20ab	26.12ab	86.02ab
0.25**	17.23a	26.77a	17.37ab	26.33ab	87.70ab
0.50**	17.18a	27.00a	17.98a	27.23a	89.39a
1.0**	18.22a	27.53a	18.21a	27.89a	91.85a
Black honey	14.50c	24.33c	16.23c	23.12c	78.18c
LSD 0.05	1.167	1.204	1.012	1.420	3.030

Within same column, means with the same letter are not significantly different; * without enzymes addition; ** concentration (ml/100g) of pectinase and cellulase.

in pH were observed. The glucose and fructose contents analysed by HPLC showed that Reziz date syrup contains equal amounts of glucose and fructose before and after addition of the enzyme mixture (37.50 vs 37.20% and 40.45 vs 39.15% respectively). These results coincide with the results of Al-Eid (2006).

Organoleptic evaluation of date syrup

The prepared date syrup prepared at ratio 3:1 water: date pulp with pectinase and cellulase at different ratios (0.25, 0.50 and 1.0%) were palatability tested in terms of color, taste, consistency and acceptability compared with syrups extracted without enzymes and sugar cane syrup (black honey), the results are listed in Table 11. It was evident from these data that syrup prepared at 1.0% of pectinase and cellulase recorded the highest average scores for color, acceptability and total score; followed by 0.5 and 0.25% and control date syrup (without pectinase and cellulase). It was clear that the cane syrup (black honey) recorded the lowest scores of all sensory evaluation terms. There were no significant differences among all studied date syrups in their color, taste, consistency and total score (Table 11). On the other hand, there were significant differences among all date syrups and cane syrup in their organoleptic properties. The results stated that, prepared date syrups are highly desirable and more acceptable than cane syrup (black honey). The results obtained are in accordance with those obtained by Al-Saidy et al. (1982), Benjamin et al. (1982), Al-Saady and Benjamin (1983) and Ramadan (1995).

Conclusion

To maximize the sugar extraction rate for about 80% or more, pectinase and cellulase (1.0%) were added to date pulp and mixed with water in ratio 3: 1 and incubated at 40°C for 24 h.

The composition and sensory evaluation of Reziz date syrup confirmed that, it was rich in sugar and minerals, has high acceptability and good expected storage ability.

Reziz date syrup contains almost equal amounts of glucose and fructose before and after the addition of enzymes mixture.

It can be concluded that, there is a good potential and the possibility to produce syrup from Reziz date fruits for use as a replacement for sucrose in food products.

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