

Effect of Enzymatic Treatments on Physico-chemical Properties and Quality of Juice and Syrup of Some Date Fruits

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Abstract

This work aims to benefit from low quality fruits (of Saidy, Tamr and Manthor dates) by the production of enzymatically treated syrup with high quality value. From the chemical analysis, the total and reducing sugars were the predominate components in all studied date fruits varied from 50.5 to 81.8% and from 40.5 to 57.7% dry weight basis (dwb), respectively. The total acidity (as malic acid) of the studied date fruits ranged from 0.14 to 0.22%.

The effect of different concentrations of pectinase enzyme, 0.5-2.0 % on the efficiency of date juice extraction process and physico-chemical proprieties of the obtained date syrup were studied. The enzyme treated date juices recorded the lowest values of viscosity, turbidity and color (as absorbance at 520 nm), with the highest values of clarity compared with that of the untreated (control) samples. The results indicated that using 1% concentration of pectinase for 60 min at 50 °C gave a good result in terms of clarity, color and turbidity, but the recovery of total soluble solids was the highest after 90 min incubation time. The color values (as optical density at 420nm) of the enzyme-treated date syrups, Saidy, Tamr and Manthor ranged from 0.499 to 0.574 compared with that of untreated date syrups which varied from 1.069 to 1.495. The treated Saidy date syrup had the highest clarity value followed by Tamr and Manthor syrups. From the obtained results it can be concluded that the enzymatic treatments could be used to produce date syrup (Dibs) with good properties and high quality from second grade date fruits.

Keywords: Low quality Date fruits, pectinase enzyme, physico-chemical proprieties, Juice, Dibs.

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, *et al.*, 1983). The Arab countries produce about 74.5% of global date

production. Egypt considered as the first country of the top ten date producers in world (FAO, 2015).

There is approximately two million tons per year, as wastes (fruits with imperfect appearance, secondary varieties not suitable for human consumption, and by-products from date processing) (FAO, 2015).

Some researchers focused on the preparation of date syrup (Al-Farsi, 2003, Al-Farsi *et al.*, 2007, Fatma *et al.*, 2011 and Enssaf *et al.*, 2013). However, there are few studies dealing with this subject and are mainly limited to traditional processes. Pectinolytic and cellulolytic enzymes are used for the fruit processing industry to increase the extraction yield, reducing sugars, soluble dry matter and titrable acidity of the products from some fruits such as peaches, plums and apricots (Joshi *et al.* 1991). In the literatures, only few works have been reported regarding the effect of hydrolytic enzymes in the quality of date syrup (Al-Hooti *et al.*, 2002). The low quality dates are a rich source of carbohydrates (73.3-89.55 g/100 g dry matter) composed mainly of sugars and dietary fibers and are safe for human consumption (Besbes, *et al.*, 2009). Owing to their composition, low quality dates were also used for preparation of some products with high value-added. The production of high quality date syrups using enzymatic treatment could give high value addition to the low quality dates (with hard texture). The aim of this work was to investigate the effect of enzyme (pectinase) treatments on composition and quality of date juice during the extraction process and to optimize process conditions. Furthermore, the effect of enzyme treatments on physico-chemical properties of the obtained date syrup was studied.

2 Materials and Methods

2.1 Materials

Dates samples: The second grade of date palm (*Phoenixdactylefera*L.) fruits namely, Saidy and

Tamer as well the seedling (Manthor) fruits were obtained from the local market of El Kharja Osis – The New Vally governorate - Egypt, during the 2016 season, date fruit samples were collected at full maturity “Tamr stage”.

Each of these fruits type was divided to three parts. The first part was used for some physical evaluations and the second part was directly cleaned, pitted and minced by meat mincer (Moulinex, type NE 401, France) before chemical analysis. The third part was prepared as above to obtain the date fruit paste and stored in polyethylene bags (about 500 g) at $4 \pm 1^{\circ}\text{C}$ until use in other treatments.

Enzyme and reagents: Commercial pectinase enzyme from *Aspergillusniger* was purchased from Sigma-Aldrich. All other reagents used in these experiments were purchased from El-gomhouria Co. (Assuit - Egypt) and the redistilled water was used.

2.2 Procedure

2.2.1 Extraction of date juice by Enzyme treatments

The date paste was homogenized with water; the pH was adjusted to 4 using citric acid, before the addition of pectinase enzyme. Samples were mixed with enzyme concentrations of 0.5, 1, 1.5 and 2 % and placed in a water bath at 50°C for 60, 90 and 120 min. Then the enzyme was deactivated by heating at 90°C for 5 min. and the date juice was extracted by a filtering cloth.

2.2.2 Production of date syrup

The clarified juices were concentrated by Rotary evaporator appa-

ratus under similar conditions (70 °C under vacuum of 500 – 600 mm/Hg) to produce date syrup of 70 °Bx.

2.3 Analytical methods

2.3.1 Physical characteristics of fruits

The studied date fruit samples were prepared for general and physical inspections by dry cleaning and sorting as following: three samples were taken randomly and physically evaluated for insect infestation percentage, number of fruits per Kg, mean weight of whole fruit, flesh and seed, fruit length and width at maximum circumference and seed length.

2.3.2 Chemical composition

Moisture, reducing – and total sugars, ash, crude protein, fiber and lipids were determined according to AOAC (2005). Non-reducing sugars were calculated by difference.

2.3.3 Pectin content:

The pectic acid content of date fruits flesh was determined by using Carry and Hayenz method (AOAC 2000). The diluted date extract treated by CaCl₂ to precipitate Ca-pectate. Pectic acid was determined as dry Ca-pectinatex 0.9235.

2.3.4 Tannins assay:

Tannins in date flesh were determined according to Lowenthal-Procter method as applied by Tafti and Fooladi (2006). The diluted extract of date fruits was titrated by vs 0.1N KMNO₄ in the presence of Indigo Carmine indicator. A blank (free of tannins) was prepared by treating the diluted extract with gelatin/charcoal was also titrated vs 0.1N KMNO₄ to correct for the actual tannin content.

2.3.5 Viscosity:

The relative viscosity of date juice was measured using an Ostwald capillary tube and the values were expressed as flow time (sec) at 25 °C. The relative viscosity was calculated by the following equation.

$$\text{Relative viscosity } \eta_r = \frac{\eta}{\eta_s}$$

Where; η = flow time of sample
 η_s = flow time of water

2.3.6 Clarity:

Clarity was measured in date juice and the reconstituted juice from date syrups according to the method described by Endo (1965). The absorbance was measured at 660 nm and clarity was expressed as transmittance value (%T).

2.3.7 Turbidity:

The turbidity index determinate according to ICUMSA method GS7-21 (1994).

2.3.8 Color:

The color was measured according to the method of Meydav *et al.*, (1977) as follows: The sample date extract produced at room temperature (25°C) by blender was centrifuged at 3300 rpm for 20 min. and the supernatant was diluted with an equal volume of alcohol (95%) and filtered through Whatman paper No. 42.

The color was measured against alcohol (95%). Browning was calculated as follows:

Browning = Absorbance of diluted sample (5% T.S.S.) at 420 nm.

2.3.9 TSS:

Abbe refractometer was used to determine the TSS of samples according to A.O.A.C. (1990) at room temperature after calibration of the scale of the refractometer.

2.3.10 pH:

The pH value was measured using a Beckman pH meter with a combination electrode at 25°C as described in the A.O.A.C. (1990).

2.3.11 Total acidity:

Total acidity (as malic acid) was determined according to A.O.A.C. (1990)

3 Results and Discussion

3.1 Physical characteristics

Data of some Physical characteristics of the studied date fruits were determined, and the results were presented in Table (1). Data showed that, Saily date fruits was the highest in the Fruit weight (g), Flesh weight (g), Flesh/pits ratio, Fruit length

(mm) and fruit diameter(mm)values compared with the other studied date fruits. Tamr date fruits were the highest in pit weight (g), Flesh (%) and Infestation (%). Furthermore, Manthor date fruits were superior to all other varieties in fruits number/ Kg and Pit (%). These differences were principally due to the hereditary aspects of palm tree, environment and the harvest time. These results were in agreement with those reported by Ramadan (1990, 1995 and 2000), El-Salhy (2000), Abdallah (2002), Osman (2008), Abd-Ellah (2009), Marzoukand Kassem (2010), Selim (2012) and Ramadan *et al.*(2016).

Table 1. Physical characteristics of the studied date samples.

Characteristics	Saily	Tamr	Manthor
Fruits number/ Kg	102	159	300
Fruit weight (g)	13.30	6.80	3.70
Flesh weight (g)	9.22	5.68	2.85
Pit weight (g)	1.34	1.38	0.80
Flesh/pits ratio	6.90	4.10	3.60
Flesh (%)	69.30	83.5	77.00
Pit (%)	10.10	20.30	21.6
Fruit length (mm)	3.30	3.10	3.00
Fruit diameter(mm)	1.90	1.70	1.60
Infestation (%)	2.00	11.00	6.00

3.2 Chemical composition of the studied date fruits

The gross chemical composition of studied date varieties was showed in Table 2. The moisture contents of Saily, Tamr and Manthor fruits were 13.21, 10.72 and 8.12% (Table 1), respectively. These results are in agreement or even less than that reported by Ramadan (1990 and 1995), Hussein *et al.* (1999) and Hasnaoui *et al.* (2010). The moisture content of Saily fruits was less than the recorded average in semi-dry

varieties, this might be reasoned to a higher loss in its moisture content after harvesting during transportation, storage and marketing. Total sugars were the main components in all studied date fruits and varied from 50.5 to 81.5%. Total and non-reducing sugars present in high amounts in Saily date, while, reducing sugar was the predominant level in Tamr dates. The pectin content of date varied between 0.97 for Manthor and 1.47% for Tamr fruits. The crude protein content of

the studied fruits ranged from 1.81 to 1.93 %. Mineral contents of Saidy, Tamr and Manthor dates were determined and the results obtained that Potassium level was the predominant among the determined elements, which was found with concentration varied from 109.37 to 157.34 mg/100g of Saidy and Manthor date fruits, respectively (Table 3). Dates also contained a high

levels of sodium and magnesium with concentrations ranged from 38.05 to 89.87 mg/100g and 9.86 to 59.10 mg/100g, respectively .

These results are similar to those reported by Ramadan (1990 and 1995), Tafti and Fooladi (2006), Besbes (2009), Borchani (2010), Selim (2012) and Ramadan *et al.* (2016).

Table 2. Mean values of gross chemical composition of the studied date fruits (dwb, except moisture).

Components (%)	Saidy	Tamr	Manthor
Moisture	13.21	10.72	8.12
Total sugars	81.50	75.90	50.50
Reducing sugars	41.70	57.70	40.50
Non-reducing sugars	39.80	18.20	10.00
Crude protein	1.93	1.86	1.81
Ash	1.73	1.79	2.50
Crude lipids	1.39	1.24	1.21
Total acidity (% malic acid)	0.14	0.19	0.22
Pectin	1.05	1.47	0.97
Tannin	0.12	1.05	2.50

Table 3. Mineral content (mg/100g sample, dwb) of the studied date samples.

Elements	Saidy	Tamr	Manthor	
Macro-elements	K	109.37	142.52	157.37
	Mg	9.86	14.38	59.10
	Na	51.76	89.87	38.05
Micro-elements	Fe	15.10	11.76	16.48
	Cu	1.04	0.968	1.42
	Mn	1.59	0.645	1.04
	Zn	0.774	1.12	6.35

3.3 Effect of enzymatic extraction on date juices

The effect of pectinase enzyme concentration (0.5, 1, 1.5 and 2%) and extraction time (30, 60, 90 and 120 min) at optimization temperature of enzyme 50°C on the total soluble solids (TSS), Clarity (660nm), Viscosity, Optical density and Turbidity

of obtained Saidy fruits juice are shown in Figures 1-5.

The results of the enzyme treatment at 50°C achieved an increase in the total soluble solids of extracted juices about 7.0-9.5%, in particular, at 60 and 90 min by enzyme concentrations 1.5 and 2.0 %, respectively (Figure 1). While the concentration of enzyme 0.5% have a

lower recovery values of TSS at all varies time incubations at the same temperature. As enzyme dose increased, juice extraction improved and more TSS value could be recovered at the same time and temperature. On the other hand the TSS recovery of the extracted date juice was gradually increased (from 14.7 to 22.2%) with the extraction time increasing (from 1 to 2 h, respectively) compared with the control at the same enzyme concentration (1.5%). Al- Hooti *et al.* (2002) employed ex-

traction procedures for producing date syrup and reported that the use of pectinase/cellulose enzymes gave the highest recovery of TSS value (68%) compared with control without these enzymes (35%). Khalil *et al.* (2002) used Ultrazyme-100G for extraction of juice from Siwi date pulp at different concentrations. This was related to the degradation of the pectin in the plant cell wall matrix and in the middle lamella into galacturonic acid units and sugars (Demir *et al.* 2001 and Landbo *et al.*, 2007).

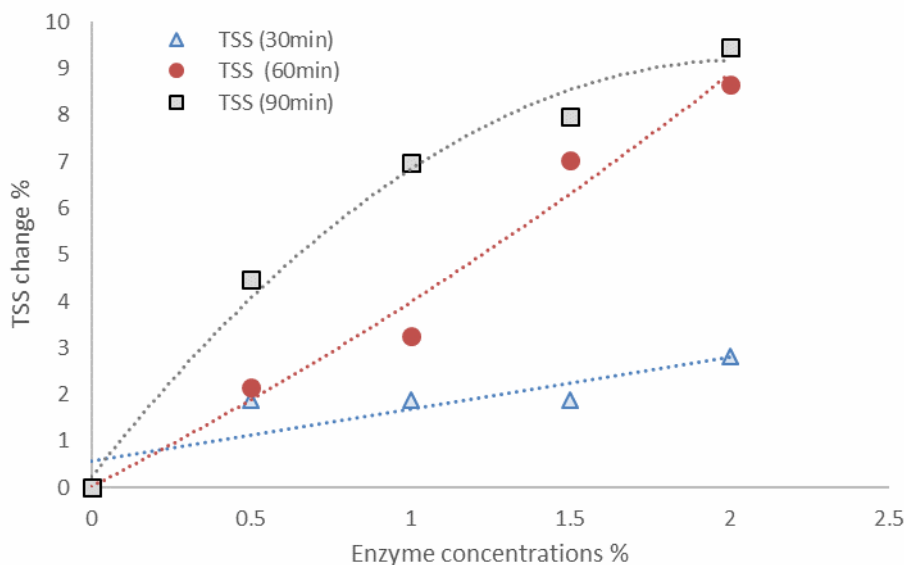


Figure (1): Effect of pectinase enzyme concentration and extraction time at 50° C on the TSS of Saida date juices.

The results indicated that enzyme treatment of date pulp greatly improved the clarity, viscosity, turbidity and color as optical density at 520 nm of the resulted juice. The results in Figure 2 showed that when the enzyme dose increased from 0.5 to 2%, clarity values of the treated samples expressed as T% at 660 nm were increased from 79.3 to 85.5%, respectively after 60 min incubation

compared with the control (50.4%). The clarity value, also increased with incubation time progress. This observation might be due to the action of pectic enzyme in breaking down the linkage between pectin and cellulose. From Figure 3, it was clear that the relative viscosity (η_r) of the produced juice gradually decreased from 0.78 to 0.45 after 60 min incubation with increasing the enzyme concentration

from 0.5 to 2%, respectively compared with the relative viscosity of untreated juice (1.31). Additionally, the relative viscosity decreased from 0.96 to 0.74 and 1.20 to 0.85 at 0.5 to 2% enzyme concentration after 90 and 120 min incubation, respectively.

The result indicated that the best enzyme concentrations were 1.5 and 2% at 60min of extraction time to improve the viscosity of the extracted date juices by decreasing about 57.6 and 66%, respectively.

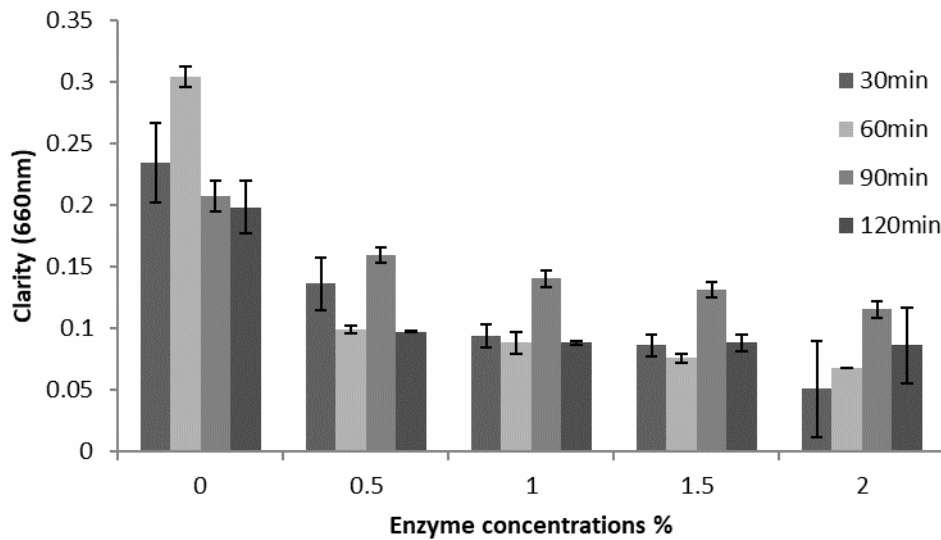


Figure (2): Effect of pectinase enzyme concentration and extraction time at 50° C on the Sady dates juice clarity (660nm).

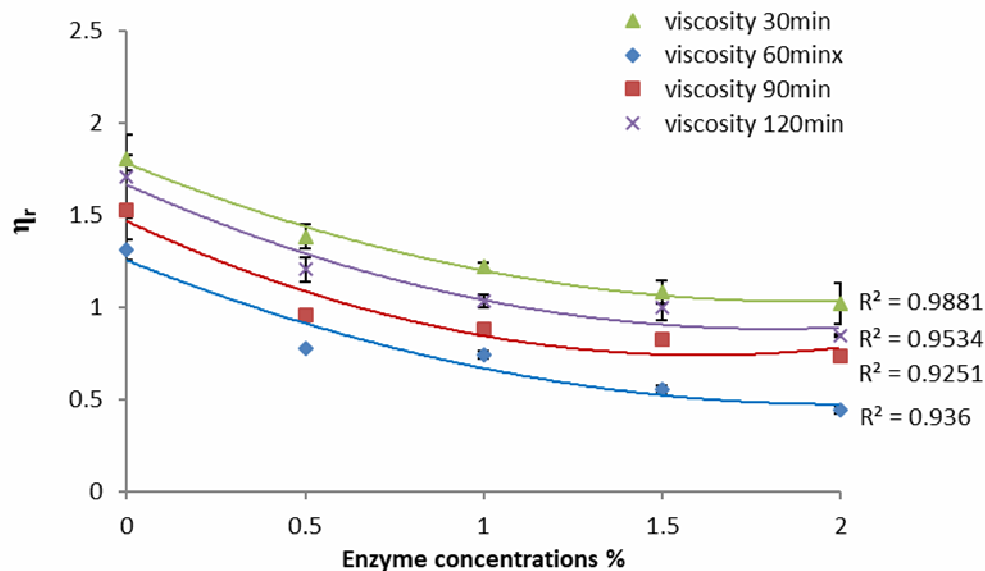


Figure (3): Effect of pectinase enzyme concentration and extraction time at 50° C on the Sady dates juice relative viscosity.

The results as shown in Figure 4 proved that enzyme treatment 0.5, 1.0, 1.5 and 2.0% of Sady date fruits

led to color removal by 56.50, 64.23, 58.17 and 66.08% (as O.D at 520nm), respectively. These results are simi-

lar to that obtained by Al-Hooti et al. (2002). As shown in Figure 5, the turbidity decreased with increase in enzyme concentration. In fact, the result of turbidity measurement indicated that the highest effect of enzyme treatment was at the extraction time of 60min of all enzyme concentration treatments. Where the turbid-

ity decreased by 66, 71, 76 and 79% of enzyme concentration 0.5, 1.0, 1.5 and 2%, respectively. The reduction of the turbidity is probably due to pectin hydrolysis in the plant cell wall matrix and in the middle lamella allowing liberation of galacturonic acid (Albagnac *et al.*, 2002).

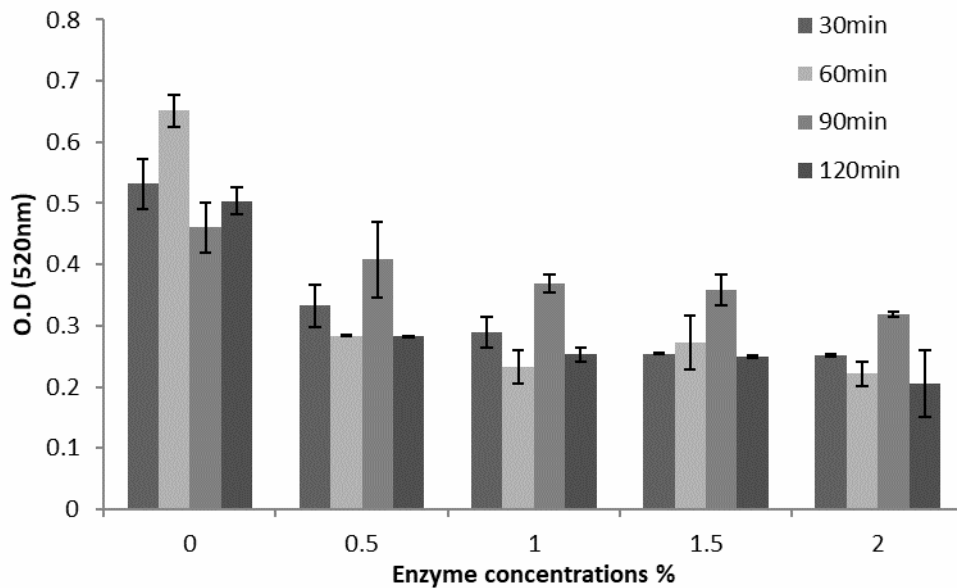


Figure (4): Effect of pectinase enzyme concentration and extraction time at 50° C on the Saïdy dates juice optical density (520nm).

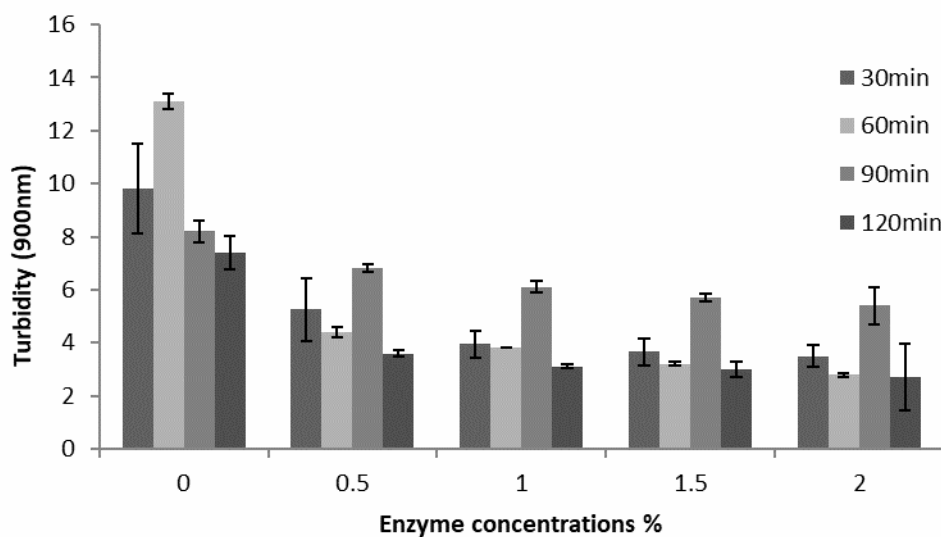


Figure (5): Effect of pectinase enzyme concentration and extraction time at 50° C on the Saïdy dates juice turbidity (900nm).

The influence of pectinase enzyme treatment, 1% at 50°C and incubation time 60 min, on the date juices characteristics of different date varieties, Saidy, Manthor and Tamr was investigated. The result was shown in Figure 6 indicated that the recovery of TSS increased by 6.0, 5.6 and 6.6% of Saidy, Manthor and Tamr, respectively. An addition, the highest effect of enzyme treatment

was in the juice clarity of Saidy (60% decrease) followed by Manthor (41% decrease) and Tamr (13% decrease) at the same condition. Also, the percentage of color removal (as optical density at 520 nm) and turbidity of treated Saidy juice were 66 and 71%, respectively. Manthor variety gave a good result of enzyme effect on juice relative viscosity (η_r) compared with Saidy and Tamr.

Table 4. Effect of enzyme treatment on physical properties of juices after 60 min of incubation and 1% enzyme concentration at 50° C for Tamr and Manthor dates fruits.

Enzyme Concentration%	TSS(°Bx)	pH	Clarity(660nm)	Viscosity	OD(520nm)	Turbidity(900nm)
Tamr						
Untreated juice	18.2	4.40	64.12	2.41	0.388	0.085
Treated juice	19.4	4.38	72.28	1.98	0.324	0.061
Manthor						
Untreated juice	18	4.37	52.6	2.96	0.612	0.117
Treated juice	19	4.33	74.3	1.89	0.345	0.049

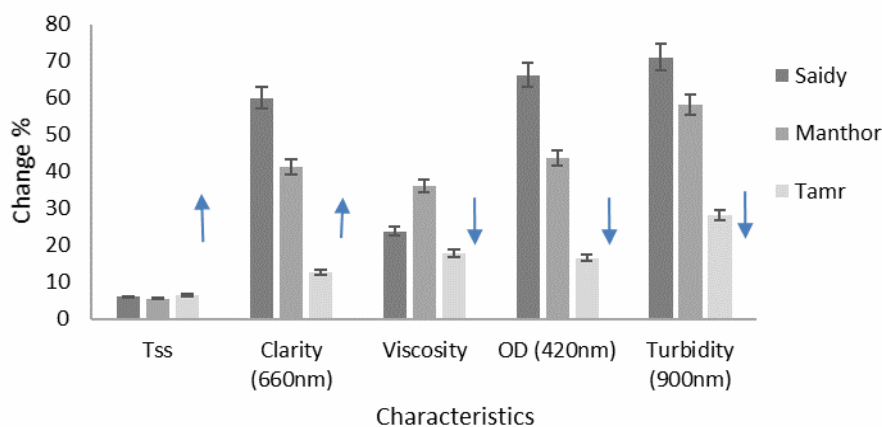


Figure (6): Effect of pectinase enzyme on physical properties of juices after 60 min of incubation and 1% enzyme concentration at 50° C for Tamr and Manthor dates fruits

3.4. Physico-chemical characteristics of dibs

Two samples of Saidy, Tamr and Manthor date syrup produced by

concentration by rotary evaporator under vacuum (treated (T_1) and untreated (T_0) with pectinase enzyme). Those samples were evaluated for

their physico-chemical characteristics. Acidity, pH, TSS, color (absorbance at 420 nm) and clarity were determined in the syrup samples, as

well as total sugars, ash, protein and total phenols content. The obtained results are presented in Table (7).

Table 5. Physicochemical characteristics of dibs produced from treated and untreated Saidy, Tamr and Manthor date juices (wwb %):

Characteristics	Saidy		Tamr		Manthor	
	T ₀	T ₁	T ₀	T ₁	T ₀	T ₁
Total soluble solids %	70.00	72.50	68.50	72.50	68.00	72.00
Total sugars %	67.86	72.00	60.43	63.00	57.69	72.67
Reducing sugars %	63.75	67.86	51	53.13	49.04	84.77
Non- reducing sugars %	4.11	4.14	9.43	9.87	7.90	8.65
Acidity (as acetic acid %)	0.39	0.90	0.59	1.59	0.79	1.10
pH	5.34	5.14	5.06	4.91	5.08	5.00
Ash %	1.70	1.50	2.60	2.40	1.90	1.70
OD (A at 420 nm)	0.77	0.52	1.45	0.57	1.06	0.49
Clarity (A at 660 nm)	0.22	0.1	0.58	0.14	0.82	0.16
Total phenols mg/g	104.97	57.25	136.07	93.57	247.31	91.29

The mineral composition of date syrup was dominated by sodium (436.99- 244.97-420.7 mg/100g) for the enzyme-treated date syrup (T₁) of Saidy, Tamr and Manthor dates, respectively and (53.71, 49.16 and 80.52mg/100g wwb) for control samples (T₀) followed by potassium (413.33 – 362.42 mg) for (T₁) and (237.61-511.01-822.42 mg/100g) for

(T₀), calcium and phosphorus. The results are in the line with those reported by Al-Hooti *et al.* (2002) who found that, sodium concentration was the greatest followed by potassium, calcium, and magnesium as the second. However, Fatma *et al.* (2011) found that potassium was the greatest followed by calcium, phosphorus and magnesium.

Table 6. Mean values of mineral contents (mg/100g sample, wwb) of the studied date syrups.

Elements		Saidy		Tamr		Manthor	
		T ₀	T ₁	T ₀	T ₁	T ₀	T ₁
Macro-elements	K	237.61	413.33	511.01	362.42	822.42	675.01
	Ca	43.99	56.13	75.68	111.60	107.12	80.73
	Mg	46.47	49.89	45.37	50.06	47.56	44.89
	Na	53.71	436.99	49.16	244.97	80.52	420.7
Micro-elements	Fe	10.48	10.19	10.52	10.77	11.02	13.50
	P	76.69	71.01	74.37	129.46	88.95	84.78
	Mn	0.58	0.82	1.82	0.59	0.52	0.51
	Zn	0.56	0.75	0.69	1.00	0.59	0.72

T₁: Enzyme treated samples

T₀: Untreated samples (control)

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تأثير المعاملات الإنزيمية علي الخواص الفيزيوكيميائية وجودة العصير والدبس الناتج من ثمار بعض التمور

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المخلص

تهدف هذه الدراسة إلى الإستفادة من الثمار المنخفضة الجودة (من البلح الصعيدي، التمر والمنتور) في إنتاج الدبس عالي الجودة بإستخدام المعاملات الإنزيمية. ومن التحليل الكيميائي للثمار إتضح أن السكريات الكلية والمختزلة تمثل المكون الرئيسي في كل عينات البلح، حيث تراوحت نسبتها من ٥٠,٥ إلى ٨١,٨ % ومن ٤٠,٥ إلى ٥٧,٥ % على التوالي. وتراوحت نسبة الحموضة (كحمض ماليك) للثمار بين ٠,١٤ إلى ٠,٢٢ % .

وقد تمت دراسة تأثير التركيزات المختلفة من إنزيم البكتينيز (٠,٥ إلى ٢%) على خواص عصير البلح المستخلص وكذلك التأثير على الخواص الفيزيوكيميائية للدبس الناتج، وقد إتضح من النتائج المتحصل عليها أن العصير الناتج بعد المعاملة الإنزيمية سجل أقل القيم من حيث اللزوجة، العكارة واللون وكان أكثر شفافية مقارنة بالعصير الناتج بدون معاملة (الكنترول). كما وجد أن أفضل النتائج من حيث الشفافية، اللزوجة، العكارة واللون للعصير الناتج كانت بإستخدام الإنزيم بنسبة ١% والتحضين لمدة ٦٠ ق على درجة حرارة ٥٠° م، وإن كانت أعلى نسبة إسترجاع للمواد الصلبة بعد التحضين ٩٠ ق.

وقد لوحظ تحسن لون الدبس الناتج من العصير المستخلص من ثمار البلح تحت الدراسة بالمعاملة الإنزيمية حيث تراوح من ٠,٤٤٩ إلى ٠,٥٧٤ مقارنة بالعينات الكنترول والتي تراوحت من ١,٠٦٩ إلى ١,٤٩٥، وقد تحسنت درجة الشفافية لعينات الدبس. ومن النتائج المتحصل عليها وجد أنه يمكن إستخدام المعاملة الإنزيمية لإنتاج دبس ذو خواص وجودة عالية من ثمار البلح منخفضة الجودة.