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RESEARCH ARTICLE

Syrup from Common Date Variety (*Phoenix dactylifera* L.): Optimization of Sugars Extraction and their Quantification by High Performance Liquid Chromatography

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Abstract: ***Background*:** In Algeria, important quantities of secondary date variety (*Phoenix dactylifera* L.) are generated in each campaign; their chemical composition is similar to that of commercial dates. The present work aims to valorize this common date variety (Degla-Beida) which is often poorly exploited.

***Methods*:** In this context, we tried to prepare syrup from the secondary date variety and evaluate the effect of conventional extraction (CE) or water bath extraction (WBE) and alternative extraction (microwaves assisted extraction (MAE) and ultrasound-assisted extraction (UAE)) on its total sugar content (TSC), using response surface methodology (RSM). Then, the analysis of individual sugars was performed by high performance liquid chromatography (HPLC).

***Results*:** Maximum predicted TSC recoveries under the optimized conditions for MAE, UAE and CE were 233.248 ± 3.594 g/l, 202.889 ± 5.797g/land 233.535 ± 5.412g/l, respectively, which were close to the experimental values: 233.796 ± 1.898 g/l; 202.037 ± 3.401 g/land 234.380 ± 2.425 g/l. HPLC analysis revealed high similarity in the sugar composition of date juices obtained by MAE (60.11% sucrose, 16.64% glucose and 23.25% fructose) and CE (50.78% sucrose, 20.67% glucose and 28.55% fructose), although a large difference was detected for that obtained by UAE (0.00% sucrose, 46.94% glucose and 53.06% fructose).

***Conclusion*:** Microwave-assisted extraction was the best method for the preparation of date syrup with an optimal recovery of total sugar content. However, ultrasound-assisted extraction was the best one for the preparation of date syrup with high content of reducing sugars.

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1. INTRODUCTION

The analysis and exploitation of bioactive substances involve their extraction from the cell matrix. Indeed, the latter are released from the vacuolar structures in which they are located, either by rupture of the plant tissue or by simple diffusion [1]. The best extraction technique is that which extracts more substances, while preserving their structure and taking a short time. The conventional extraction process is commonly used [2], although recently many non-conventional methods, which are rapid and automated, have been used, *e.g.* supercritical fluid extraction (SFE), pressurized liquid extraction (PLE), microwave-assisted extraction (MAE) and ultrasound-assisted extraction (UAE) [3]. Many reports have confirmed that ultrasound and microwave extractions are the fastest methods for extracting bioactive components from vegetable matrices [2, 4].

Since 7000 years, dates have played an important role in human life [5]. They are the fundamental fruit of oasis ecosystems, and have been an important crop in the Sahara regions [5-7]. The industrialization and transformation of dates are gradually increasing due to their richness in essential nutrients [5]. The date and its by-products play an important role in the economy of producing countries [6, 8]. During the Ramadan period, dates are the main fruits for the Algerians and especially for the inhabitants of the Sahara [9]. They are used with fermented milk to break the fast because they are considered an important source of nutrients and energy.

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Palm date fruits and syrups are used to treat liver diseases in traditional medicine [10]. Because of their richness in potassium and its low sodium content, they are considered as a desirable food for hypertensive patients who are advised to consume low sodium diets [11]. The regular consumption of dates is beneficial for the treatment of cough, rheumatism, burning sensation, nephropathy, gastropathy, and sexual debility. Therefore, they are prescribed for gastroenteritis, respiratory diseases, asthma, chest complaints, fevers and high blood pressure [10]. Epidemiological studies have also reported their effect on reducing the risk of several neurodegenerative diseases, such as tumors and mutagens. They are also used to stimulate the immune system of women after giving birth [12].

The global production of date fruits exceeds 7 million metric tons annually in the world. Algeria is the second producer in the world with an annual production of 720 000 tons [13], and the first producer in the Arab Maghreb union [14]. Cultivars with a low commercial quality, known as common cultivars, are discarded and account for approximately 30% of the Algerian production [15], although second-grade dates (with a hard texture) contain the same levels of sugar, fiber and total phenols [7].

Date syrup (date honey), is a dark sweet syrup obtained from the date extract [6] and it is made with different varieties of dates, preferably those of secondary quality [16, 17]. It is a highly concentrated liquid [18], which is a naturally inverted sugar, with equal proportions of glucose and fructose, and a small amount of sucrose, which under the effect of temperature and pH conditions (acid medium) can be inverted to a simple sugar [16]. It is consumed alone as honey, or used as an ingredient in food recipes such as: ice cream, beverages, confectionery, pancakes and waffles.

The chemical composition and nutritional value of date syrup have been well studied [6, 8, 19]. Date syrup is rich in carbohydrates and minerals, saccharides, amino and organic acids, polyphenols and carotenoids [6, 9]. It can replace synthetic sweeteners [17], which during the manufacturing processes can lead to the formation of toxic products [20]. As it is a natural invert sugar, toxic chemicals are not produced after its manufacturing processes (pressing, extraction at low and high temperature) [16].

The main objective of this work is to optimize the method of preparation of syrup with high total sugar content (TSC), from low quality and hard texture (Degla-Beida) dates. To this end, three methods were proposed: microwave method (MM), ultrasound method (UM) and conventional or water bath method (CM or WBM). Response surface methodology (RSM) was used to evaluate the factors affecting the sugar yield in both methods. The optimized extracts were then analyzed for their individual sugar composition using HPLC.

2. MATERIAL AND METHODS

2.1. Chemicals and Sample Treatment

All the chemicals used in this study were purchased from Sigma-Aldrich (represented by Algerian Chemical Society, Setif, Algeria). The date used was a common variety called Degla-Beida, of dry nature and hard texture, from Biskra (Algeria) at the final stage of fruit ripeness. The samples were washed with tap water followed by distilled water, pitted and deposited in a drying oven at 40°C until an unvarying weight and then grounded into a fine powder; this powder was passed through a standard 125 µm sieve and only the fraction with particle size <125 µm was used. The powder was stored in the dark until use. The choice of this variety is justified by its relative abundance in Algeria, low-market value, long-term conservation for its dry nature and by its taste and nutritional quality (source of energy), especially represented by its sugar content.

2.2. Preparation of Date Juices

Three extraction methods were used for the preparation of date juices: microwave-assisted extraction (MAE), ultrasound-assisted extraction (UAE) and conventional extraction (CE).

To prepare date syrups, the obtained juices were concentrated at 70°C using an oven until having syrups at 80°Brix.

2.2.1. Microwave Assisted Extraction (MAE)

A microwave oven (MAXMOS23S, Maxipower, China) working at 2450 MHz frequency with cavity dimensions of 28.1cm (H) × 48.3 cm (W) × 38.7 cm (D) was used. Ten grams (10 g) of date powder was mixed with 60, 70, 80, 90, 100, 120 and 140 ml of distillated water. The samples were treated under microwave irradiations in an intermittent way, *i.e*. irradiation-cooling-irradiation at power input of 100, 300, 500, 700 and 900 W with fixed irradiation and cooling time of 0.30, 0.45, 1, 2, 3, 4 and 5 min. After irradiation, the extract was filtered through a Whatman filter paper (n° 4), and centrifuged (NF200, NÜVE, Turkey) at 4000 rpm for 15min, and then adjusted to 100 ml with distilled water. Finally, the extract was stored at 4°C until use.

2.2.2. Ultrasound Assisted Extraction (UAE)

Date powder (10g) was immersed into 60, 70, 80, 90, 100, 120 and 140 ml of distilled water. The extraction process was performed using an ultrasonic cleaner (2510E-DTH, Mexico, USA), with working frequency fixed at 42 kHz and different ultrasonic temperatures: 30, 40, 50, 60 and 69°C. After 15, 30, 45, 60, 90 min, the extract was filtered through Whatman filter paper (n° 4), centrifuged (NF200, NÜVE, Turkey) at 4000 rpm for 15 min, and then adjusted to 100 ml with distilled water. At the end, the extract was stored at 4°C, until use.

2.2.3. Conventional Extraction (CE) or Water Bath Extraction (WBE)

Conventional extraction was carried out as follows: 10g of date powder was extracted for 1, 30, 60, 90, 120 and 180 min using a thermostatic water bath shaker (WNB22, Memmert, Germany) with 60, 70, 80, 90, 100, 120 and 140 ml of distilled water at different shaking speed (30, 50, 70, 90 and 110rpm, respectively) and different temperatures (40, 50, 60, 70, 80, 90 and 95°C, respectively). The extract was filtered

through Whatman filter paper (n° 4), centrifuged (NF200, NÜVE, Turkey) at 4000 rpm for 15 min, and then adjusted to 100 ml with distilled water. The obtained extract was stored (4°C) until use.

2.3. Determination of Total Sugar Content (TSC)

The most commonly used method to measure carbohydrate concentrations is the anthrone method [21]. Following the method described by Hanson and Phillips, (1981) [22], briefly, 400 µl of the extract (diluted in distilled water) was placed in the test tube, 2 ml of anthrone reagent was added and the solution was mixed. After incubation at 100°C for 10 min, the absorbance of the sample was measured at 625 nm against a blank (made as reported for the sample but with 400 µl of distilled water), by using a UV-vis Spectrophotometer (SpectroScan 50, UK). Glucose was used as standard for the calibration curve to express the TSC concentration of the sample as mg Equi. Glu/ml.

2.4. Identification of Sugars From Date Syrups by HPLC

High performance liquid chromatography was used for the analysis of sugars (fructose, glucose and sucrose) from the optimized juices. The separation was carried out on a cationic (Ca^{++}) or cation exchange column, the mobile phase (the eluent) was the distilled water and the detector was a Refractive Index Detector (RID). Sugars were extracted from different samples (0.1g) by stirring for 10 min in a 100 ml flask with 20 ml of distilled water, and then adjusted to 1.5ml. The samples were filtered through membrane filters (0.45 µm) and analyzed by liquid chromatography (LC). The results are expressed in percentages (%) by the software calculation of the area of each peak. The molecules of glucose, fructose and sucrose were characterized by their retention time (by comparison with their respective standards).

2.5. Experimental Design and Statistical Analysis

Response surface methodology (RSM) was applied to determine the optimal processing conditions generated by the statistical software package JMP (10.0.0 version, SAS Institute, USA). Three extraction methods with different processing variables including: X_1-Microwave power, X_2-Irradiation time, and X_3-Liquid/solid ratio for MAE; X_1-Ultrasonic temperature, X_2-Ultrasonic treatment time and X_3-Liquid/solid ratio for UAE and X_1-Temperature, X_2-Treatment time, X_3-Liquid/solid ratio and X_4-Shaking speed for CE, were chosen based on the results of the single factor experiments (Table **1**). Then, the effects of these key variables were investigated using Box-Behnken experimental design (BBD) in RSM to develop a response surface quadratic model for describing the extraction process. Then the yield of TSC (Y) was taken as the response of the design experiments. According to this design, a total of 15 experiments were performed for MAE and UAE; however, 27 experiments were performed for WBE (Table **2**) containing three replicates at the center point. The selected variables were coded according to the following equation:

$$x_i = X_i - X_{0i} / \Delta X \quad (1)$$

where x_i and Xi are the least coded dimension and the actual values for its independent variables, respectively, X_0 is the actual value for the independent variable at the center point, and ΔX is the step change value.

The regression analysis of the experimental data was performed to establish the empirical second order polynomial models, shown in Eq 2:

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X^2 + \sum_{ij}^{k} \beta_{ij} X_i X_j + E \quad (2)$$

where Y is the measured response variable, which is in our case the total sugar content (TSC), $ß_0$ is a constant, $ß_i$ is the linear coefficient (main effect), $ß_{ii}$ is the quadratic coefficient, $ß_{ij}$ is the two factor interaction coefficients, and X_i, X_j are the independent variables.

Each extraction trial and all the analyses were carried out in triplicate and all the data in this paper were reported as means ± standard deviation (SD). The influence of each factor on the TSC yield in the single factor experiment for the MAE, UAE and CE was statistically assessed by ANOVA analysis of variance and Tukey's post hoc test with 95% confidence level using XLSTAT Release 10 (Addinsoft, Paris, France).

3. RESULTS AND DISCUSSION

Before carrying out the BBD approach, preliminary experiments were undertaken to select the lower, middle and upper level (+1, 0, -1) for each factor. The results of the preliminary experiments for the BBD are shown in Table **1**. Distilled water was then selected as the solvent for the RSM trials and it was used for all the extraction methods.

3.1. Microwave Assisted Extraction (MAE)

3.1.1. Effect of Independent Variables on TSC Yield

Low microwave power reduces extraction efficiency. However, too much power results in wasted energy; therefore, the optimal microwave power should be determined. TSC significantly increased when the microwave power increased from 100 W to 500 W, whereas the other extraction parameters were constant (1 min irradiation time and 10:1 ml/g liquid/solid ratio). TSC recovery was parabolic with a maximum value at 500 W. However, as the microwave power continued to increase up to 900 W, TSC decreased. Based on these results, the microwave power range 300-700 W was selected for the RSM trials and 500 W was fixed for the next single factor experiments on the influence of microwave irradiation time.

Extraction time is an important parameter in the extraction of phytochemicals. It has two phases: 1) the dissolution of soluble components on the surface of the sample particles, and 2) the mass transfer of the solute from the plant matrix into the solvent by diffusion and osmotic processes [23]. When the contact time between the solvent and the sample is longer, its absorption is improved, and the permeability of the cell wall is increased, which allows the solvent to penetrate the plant material in order to accelerate the release of

Table 1. Results of single-factor experiments of MA, UA and WB extractions.

MAE						UAE						WBE							
Power		Time		Ratio		T°		Time		Ratio		T°		Time		Ratio		Shaking Speed	
W	TSC (g/l)	s	TSC (g/l)	ml/g	TSC (g/l)	°C	TSC (g/l)	min	TSC (g/l)	ml/g	TSC (g/l)	°C	TSC (g/l)	min	TSC (g/l)	ml/g	TSC (g/l)	rpm	TSC (g/l)
100	104 ±5^{c}	30	110 ±3^{d}	6	73, ±9^{d}	30	33 ±3^{c}	15	103 ±4^{c}	6	81 ±4^{c}	40	144 ±5^{b}	1	151 ±3^{d}	6	152 ±4^{c}	30	156 ±10^{b}
300	190 ±3^{b}	45	155 ±8^{b}	7	111 ±7^{c}	40	92 ±7 ^{b}	30	131 ±2^{ab}	7	94 ±4^{bc}	50	168 ±8^{b}	30	217 ±8^{ab}	7	175 ±7^{bc}	50	162 ±10^{b}
500	220 ±1^{a}	60	220 ±1^{a}	8	117 ±5^{c}	50	108 ±10^{b}	45	153 ±6^{bc}	8	120 ±5^{abc}	60	180 ±8^{b}	60	229 ±3^{b}	8	182 ±5^{ab}	70	177 ±7^{ab}
700	175 ±4^{b}	120	230 ±1^{a}	9	160 ±6^{b}	60	131 ±10^{a}	60	196 ±6^{a}	9	154 ±6^{ab}	70	189 ±4^{ab}	90	174 ±6^{bc}	9	205 ±3^{ab}	90	195 ±6^{ab}
900	118 ±4^{c}	180	205 ±5^{a}	10	182 ±8^{a}	69	131 ±10^{a}	75	165 ±5^{a}	10	190 ±9^{a}	80	202 ±5^{ab}	120	160 ±4^{cd}	10	216 ±4^{a}	110	229 ±3^{a}
		240	156 ±7^{c}	12	184 ±6^{a}			90	119 ±7^{bc}	12	191 ±7^{a}	90	217 ±8^{a}	180	155 ±6^{cd}	12	215 ±6^{a}		
		300	132 ±4^{c}	14	185 ±7^{a}					14	190 ±5^{a}								

Values are mean ± 95 % confidence interval.
Values with different letters (a-b-c) were significantly different (Tukey, $p < 0.05$).

Table 2. Box-Behnken design with the observed responses of total sugar content (TSC) yield from (*Phoenix dactylifera L.*), using MA, UA and WB extractions.

Run	MAE					UAE					WBE					
	X_1	X_2	X_3	TSC (g/l)		X_1	X_2	X_3	TSC (g/l)		X_1	X_2	X_3	X_4	TSC (g/l)	
				Actual	Predicted				Actual	Predicted					Actual	Predicted
1	300	2	10	212.22 ±0.74	211.47	40	60	10	186.17 ±2.04	187.24	75	60	70	10	219.14 ±0.77	216.34
2	500	3	10	228.27 ±2.04	230.21	60	60	8	178.15 ±1.96	177.08	75	30	90	7	184.44 ±3.16	184.87
3	300	1	9	202.35 ±1.19	202.81	50	30	8	161.11 ±1.34	162.69	90	60	90	10	228.64 ±0.93	230.38
4	300	2	8	176.42 ±2.04	177.89	50	90	8	155.56 ±1.11	156.67	75	60	110	10	229.75 ±2.04	228.58
5	300	3	9	199.38 ±2.41	198.21	60	90	9	189.38 ±1.19	189.34	75	90	110	8.5	194.44 ±1.70	194.35
6	500	3	8	192.47 ±3.52	192.18	50	60	9	180.99 ±0.57	179.42	60	60	90	10	206.79 ±0.57	207.87
7	700	2	8	180.00 ±2.06	180.76	60	60	10	201.11 ±0.98	202.73	75	60	90	8.5	202.22 ±1.34	201.52
8	500	2	9	224.57 ±1.30	222.59	50	30	10	190.12 ±6.02	189.01	75	60	90	8.5	204.20 ±2.41	201.52
9	500	2	9	220.49 ±2.04	222.59	40	30	9	171.36 ±4.26	171.40	75	30	70	8.5	185.56 ±1.11	186.90
10	700	2	10	222.72 ±2.26	221.25	50	60	9	180.37 ±1.28	179.42	75	90	90	7	178.52 ±1.70	178.71
11	500	1	10	230.49 ±1.50	230.79	60	30	9	191.36 ±1.30	190.85	75	30	90	10	213.46 ±2.63	214.15
12	500	1	8	196.67 ±1.48	194.74	40	60	8	154.07 ±1.96	152.45	75	90	70	8.5	184.44 ±1.70	184.89
13	500	2	9	222.72 ±2.63	222.59	40	90	9	168.15 ±1.70	168.66	75	30	110	8.5	198.15 ±3.90	198.96
14	700	3	9	208.03 ±1.67	207.56	50	90	10	192.35 ±0.86	190.77	75	60	70	7	186.67 ±1.70	185.70
15	700	1	9	204.94 ±1.90	206.11	50	60	9	176.91 ±2.99	179.42	60	60	110	8.5	185.93 ±1.11	187.19
16											90	60	90	7	209.63 ±1.96	209.80
17											60	60	90	7	164.69 ±0.86	164.21
18											90	60	110	8.5	218.03 ±2.23	216.55

(Table 2) contd….

Run	MAE					UAE					WBE					
	X_1	X_2	X_3	TSC (g/l)		X_1	X_2	X_3	TSC (g/l)		X_1	X_2	X_3	X_4	TSC (g/l)	
				Actual	Predicted				Actual	Predicted					Actual	Predicted
19											90	30	90	8.5	209.26 ±2.89	208.66
20											75	60	110	7	194.32 ±1.50	194.98
21											75	90	90	10	213.21 ±1.67	213.67
22											60	60	70	8.5	169.38 ±1.71	171.74
23											90	90	90	8.5	200.74 ±0.37	201.28
24											60	30	90	8.5	173.21 ±0.93	170.54
25											90	60	70	8.5	210.86 ±1.67	210.48
26											75	60	90	8.5	198.15 ±2.31	201.52
27											60	90	90	8.5	172.84 ±1.07	171.30

Values are mean ± 95 % confidence interval.

the compounds of interest. However, a very long time can result a decrease in the efficiency of the extraction process. Therefore, the irradiation time must be appropriate. The extraction was carried out at different irradiation times, whereas other parameters were constant (500 W microwave power and 10:1 ml/g liquid/solid ratio). The yield of TSC significantly increased as the irradiation time increased from 30 s to 2 min. Then the recovery was maximized at 2 min and declined moderately with further increases in irradiation time (5 min). Since shorter extraction time is also favorable to reduce energy costs, the range 1-3 min was selected for the RSM study, while 2 min irradiation time was used for the last single factor trials dealing with a varying liquid/solid ratio.

For efficient extraction, the solvent volume must be sufficient to ensure complete immersion of materials, and an extraction solvent deficiency can lead to lower extraction yields (incomplete extraction) of ingredients [24], although redundant solvent may also lead to lower extraction yields and solvent waste [25]. Therefore, the liquid/solid ratio must be appropriate. The extraction was carried out at different liquid/solid ratios, whereas other extraction parameters were constant (500 W microwave power and 2 min irradiation time). The extraction yield of TSC significantly increased as the liquid/solid ratio increased within the range of 6:1-10:1 ml/g. However, the extraction yield did not change significantly when the liquid/solid ratio continued to increase (14:1 ml/g). Based on the statistical analysis, the range 8:1-10:1 ml/g was selected for the RSM optimization.

3.1.2. Optimization by RSM

Fifteen experiments were carried out according to the conditions indicated in Table **2**. Response values (TSC) are reported in the last column of this table. The combined effects of the microwave power, irradiation time, and liquid/solid ratio on the yield of TSC are shown in Fig. (**1**). The relationship between the response and the experimental variables can be illustrated graphically by plotting three-dimensional response surface plots (Figs. **1a-1c**). The vertical axes show the yield of TSC, and each of the two horizontal axes represents two of the three independent variables. In every plot, the factor not represented by the two horizontal axes was fixed at its 0 coded level. As can be seen in the figure and table, all the studied factors have a significant influence on the TSC, *i.e.* a significant increase in the recovery of total sugars with the elapse of irradiation time when microwave power was increased.

Fig. (**1a**) illustrates the interaction effect of microwave power and irradiation time, when the liquid/solid ratio was set at its 0 level (9:1 ml/g). The increase in microwave power and irradiation time increased the TSC up to a maximum of 224.57 g/l. However, a prolonged extraction time with the microwave power gave reduced TSC yield, which was also noticed during our preliminary study.

The effects of the interaction between microwave power and liquid/solid ratio on the TSC when the irradiation time was set at its 0 level (2 min) are presented in Fig. (**1b**). The results indicated that the yield increased with the increase of microwave power and liquid/solid ratio at the beginning of the extraction. The recovery reached its maximum (224.57g/l) at 500 W and 9:1 ml/g liquid/solid ratio during the MAE process. The increases in microwave power above 500 W led to decreases in the extraction recovery of TSC.

The effects of the interaction between irradiation time and liquid/solid ratio at 500 W (0 level) on the extraction yield of TSC are presented in Fig. (**1c**). TSC increased roughly from 192.47 to 230.49 g/l when the ratio increased from 8:1 to 10:1 ml/g and the irradiation time varied for 1-2 min; then, the decline was observed after 2 min.

The predicted yield (233.25 ± 3.59 g/l) obtained by the optimal conditions (power of 530 W, irradiation time of 1.99 min and liquid/solid ratio of 10:1 ml/g) was very close to the value predicted by the model (233.80 ± 1.90 g/l).

3.2. Ultrasound Assisted Extraction (UAE)

3.2.1. Effect of Independent Variables on the TSC Yield

The propagation of ultrasonic waves and cavitation effects are two key factors resulting in the enhancement of the

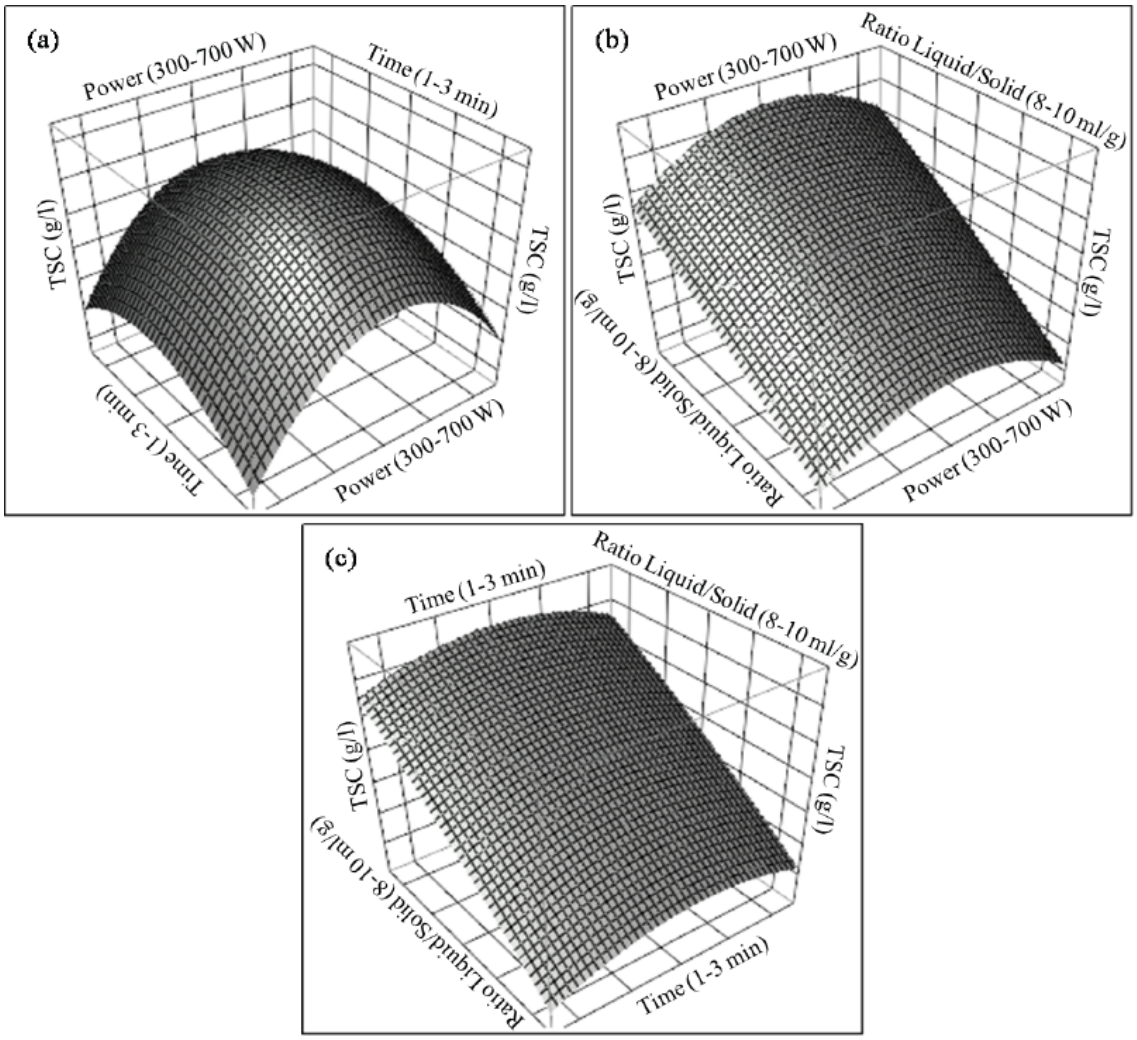

Fig. (1). Response surface analysis for the total sugar yield from *Phoenix dactylifera* L. by microwave-assisted extraction with respect to microwave power and irradiation time (**a**); microwave power and liquid/solid ratio (**b**) and irradiation time and liquid/solid ratio (**c**).

efficiency of the bioactive compounds extraction [26]. The cavitational effect, which increases mass transfer and results in close interaction between the solvent and plant tissues, is the principle of ultrasound-assisted extraction. Briefly, the implosion of cavitation bubbles generates macro-turbulence, high-velocity inter-particle collisions, and perturbation in micro-porous particles of the biomass, which accelerates the internal diffusion. Moreover, the collapse of cavitation bubbles in plant tissue surfaces produces micro-jets, leading to tissue disruption and good solvent penetration into the tissue matrix [27]. Usually, ultrasonic power is believed to be the driving force for the complete dispersion of solvent into the solid sample [28]. In this work, ultrasonic power was fixed to 42KHz. In the case of temperature, its increases can influence the membrane structure of the plant cells, making them less selective [29]. Additionally, at high extraction time, sugars can be degraded. Different temperatures were investigated (30-69°C) at a fixed ultrasonic treatment time of 30 min and 10:1 ml/g liquid/solid ratio. The TSC yield significantly increased when the ultrasonic temperature increased from 30 to 60°C. However, the extraction yields did not change significantly when the ultrasonic temperature continued to increase (69°C). Based on our observations, moderate ultrasonic temperature levels of 40, 50 and 60°C were selected as the lower, middle and upper levels, respectively, to apply in RSM optimization.

When the ultrasonic treatment time was at a low level (15 min), the yield of TSC increased to a certain value at 60 min with a fixed temperature of 60°C and 10:1ml/g liquid/solid ratio, after which, the yield decreased with increasing ultrasonic treatment time up to 90 min. The highest TSC yield was obtained at 60 min. Based on our results; the 30-60 min range was used for further experimentation using UAE for RSM optimization while 60 min was kept for the next single-factor trials.

A positive effect on TSC yield was recorded when the liquid/solid ratio was 10:1 ml/g. There was an increase in the yield with the increase of liquid/solid ratio from 6:1 to 10:1 ml/g. However, TSC was not significantly affected when the liquid/solid ratio continued to increase (14:1 ml/g). Based on the statistical analysis, the range 8:1-10:1 ml/g was finally selected for the RSM optimization.

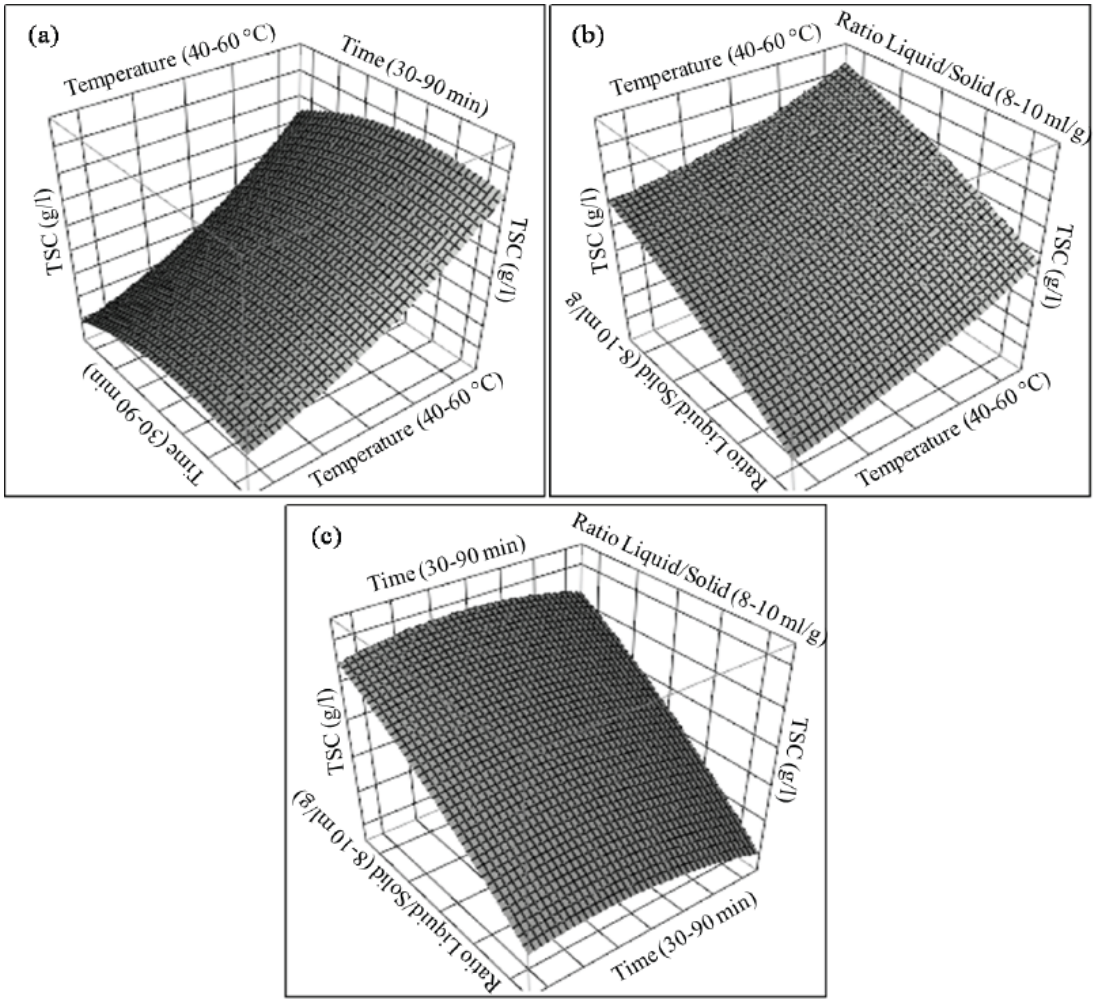

Fig. (2). Response surface analysis for the total sugar yield from *Phoenix dactylifera* L. by ultrasound-assisted extraction with respect to ultrasonic temperature and treatment time (**a**); ultrasonic temperature and liquid/solid ratio (**b**) and treatment time and liquid/solid ratio (**c**).

3.2.2. Optimization by RSM

In this optimization, we evaluated the effects of ultrasonic temperature, ultrasonic treatment time and liquid/solid ratio on TSC yield. The data obtained from 15 runs of experiments using UAE are shown in Table **2**. The three-dimensional response surface plots in Fig. (**2**) illustrate the relationship between the extraction yield of TSC and the experimental variables.

The effect of ultrasonic temperature and ultrasonic treatment time on the extraction yield of TSC at constant liquid/solid ratio (0 level) is shown in Fig. (**2a**). The extraction yield of TSC gradually increased with temperature and ultrasonic treatment time from 171.36to 191.36 g/land peaked at 60°C and 30 min. The extraction yield of TSC began to decrease beyond 60 min.

The appropriate liquid/solid ratio (10:1 ml/g) had positive effects on the extraction yield of TSC, as is shown in the response surface plots for the effect of liquid/solid ratio on the extraction yield (Fig. **2b** and **2c**) at constant ultrasonic treatment time and constant ultrasonic temperature, respectively. The extraction yields increased along with the increase in the liquid/solid ratio from 8:1 to 10:1 ml/g and ultrasonic temperature from 40 to 60°C to reach 201.11 g/l (Fig. **2b**). It was also observed that a simultaneous increase in the liquid/solid ratio from 8:1 to 10:1 ml/g and ultrasonic treatment time of 30-60 min increased the TSC yields from 178.15 to 201.11 g/l. After this increase, the TSC yields began to decrease beyond 60 min (Fig. **2c**).

The predicted yield (202.89 ± 5.80g/l) obtained by the optimal conditions (ultrasonic temperature 60°C, treatment time 68.10 min and liquid/solid ratio 10:1 ml/g) was very close to the value predicted by the model (202.04 ± 3.40 g/l).

3.3. Conventional Extraction (CE) or Water Bath Extraction (WBE)

3.3.1. Effect of Independent Variables on TSC Yield

It is known that high temperature can facilitate the release of sugars into the headspace by overcoming energy barriers that bind them to the matrix. Different temperatures were investigated from 40-90°C at a fixed treatment time of 30 min, 110 rpm shaking speed and 10:1 ml/g liquid/solid ratio. As indicated in this paragraph, most sugars were extracted efficiently at 90°C. Based on these results, the temperature range 60-90°C was selected for the RSM trials and

90°C was fixed for the next single-factor experiments on the influence of treatment time.

Treatment time is one of the most important parameters in the water bath extraction method. In this study, different extraction times were investigated, from 1-180 min, at a fixed temperature of 90°C, 110 rpm shaking speed and 10:1 ml/g liquid/solid ratio. An increase in efficiency was observed when the extraction time increased from 1 min to 60 min, and then it began to decrease beyond 60 min. Thus, the optimum equilibrium time was determined, and the range of 30-90 min was selected for the RSM trials, while 60 min was kept for the next single-factor trials.

Different liquid/solid ratios from 6:1 to 14:1 ml/g were selected to be compared for their effectiveness in TSC extraction, in terms of extraction quantity and repeatability at a fixed temperature of 90°C, 60 min extraction time and 110 rpm shaking speed. The 10:1 ml/g liquid/solid ratio presented a higher yield for the investigated compounds. Thus, based on these preliminary results, the 10:1 ml/g liquid/solid was selected for the extraction of total sugars in the subsequent experiments while the range 6:1-10:1 ml/g was selected for the RSM trials.

The addition of shaking speed as a fourth factor can increase the extraction yield. As a consequence, extraction efficiency is improved due to the favored diffusion of the analytes to the headspace. Different shaking speeds (30, 50, 70, 90 and 110 rpm) were used to investigate their influence on the conventional procedure at a fixed temperature 90°C, 60 min extraction time and liquid/solid ratio 10:1 ml/g. Increases in TSC yield were observed with the increasing of shaking speed from 30 to 110 rpm. Thus, higher level resulted in higher extraction efficiency. Therefore, 110 rpm shaking speed was chosen as the best condition in this regard while the range 70 to 110 rpm was selected for the RSM trials.

3.3.2. Optimization by RSM

The experimental design and corresponding response data for the TSC from *Phoenix dactylifera* L. for the conventional extraction are presented in Table **2**, with twenty seven experiments. The effect of temperature, treatment time, liquid/solid ratio and shaking speed on the extraction yield of TSC was investigated, and the results are shown in Fig. (**3**). The three-dimensional response surface plots in this figure illustrate the relationship between the TSC extraction yield and the experimental variables. The plots were generated by plotting the response using the z-axis against two independent variables (X_1 and X_2) while keeping the other two independent variables (X_3 and X_4) at their zero level. These results indicate that all tested parameters have a great impact on TSC extraction yield.

The profiles obtained for the effect of temperature and treatment time on TSC extraction yield at 0 levels, fixed shaking speed and liquid/solid ratio are shown in Fig. (**3a**). The extraction yield of TSC gradually increased with temperature and treatment time and peaked at approximately 90°C and 30 min to reach 209.26 g/l. Further increase in treatment time enhanced the degradation of the investigated compounds, which resulted in the decrease of the TSC to 200.74 g/l.

With respect to the interaction between temperature and shaking speed when treatment time and liquid/solid ratio were set at their 0 levels, presented in Fig. (**2b**), by increasing the temperature and the shaking speed from 60 to 90°C and 70 to 110 rpm, respectively, the TSC increased from 169.38 to 218.03 g/l and peaked at approximately 90°C and 110 rpm shaking speed. Fig. (**1d**). illustrates the interaction effect of extraction time and shaking speed, when the temperature and liquid/solid ratio were set at their 0 levels (75°C and 8.5:1 ml/g); the increase in the extraction time and shaking speed increased the TSC up to a maximum of 204.20 g/l. However, a prolonged extraction time reduced it; this result was also noticed during our preliminary study.

The response surface plots for the effect of liquid/solid ratio on extraction yield (Figs. **3c-3e-3f**) at constant temperature, treatment time and shaking speed show that an appropriate extraction liquid/solid ratio (10:1 ml/g) had a positive effect on the extraction yield of TSC (Fig. **3c**), when the 3D response surface plot was developed for the recovery of TSC with varying temperature and liquid/solid ratio. It can be observed that a maximum recovery of TSC (228.64 g/l) was achieved with a temperature of90°C at a liquid/solid ratio of 10:1 ml/g. In the latter case, the extraction was increased when the treatment time was lower than 60 min and decreased when it exceeded 60 min. The interactive terms, in the model, are not significant. Fig. (**3e**) shows that the TSC could be maximized to 213.46 g/l at about 30 min and 10:1 ml/g liquid/solid ratio over a range of the other operational factors (temperature and shaking speed). In fact, in water bath extraction, shaking speed is one of the key variables affecting the release of sugars from different matrices. The increase in the shaking speed accelerated sugar extraction. Fig. (**3f**) shows that with an increase in shaking speed from 70 to 110 rpm and liquid/solid ratio from 7:1 to 10:1 (ml/g), the extraction yield of TSC increased gradually from 186.67 to 229.75 g/l.

From the model, the optimal conditions of CE were: temperature 90°C, extraction time 55.34 min, 110 rpm shaking speed and liquid/solid ratio 10:1 ml/g with a predicted yield of 233.54 ± 5.41g/l. CE was carried out under these conditions giving a real recovery of 234.38 ± 2.43 g/l.

3.4. Verification of the Predictive Models

Based on the above findings, to validate the predictability of the established models, the optimized parameters were tested. The mean values 233.25 ± 3.59 g/l, 202.89 ± 5.80g/l and 233.54 ± 5,41g/l were found in the actual experiments for MAE, UAE and CE, respectively, which were in close agreement with the predicted values (233.80 ± 1.90 g/l, 202.04 ± 3.40 g/land 234.38 ± 2.43 g/l) using the respective model Eqs. (3), (4) and (5). The strong correlation between these results confirmed that the response models were adequate in predicting the optimized conditions.

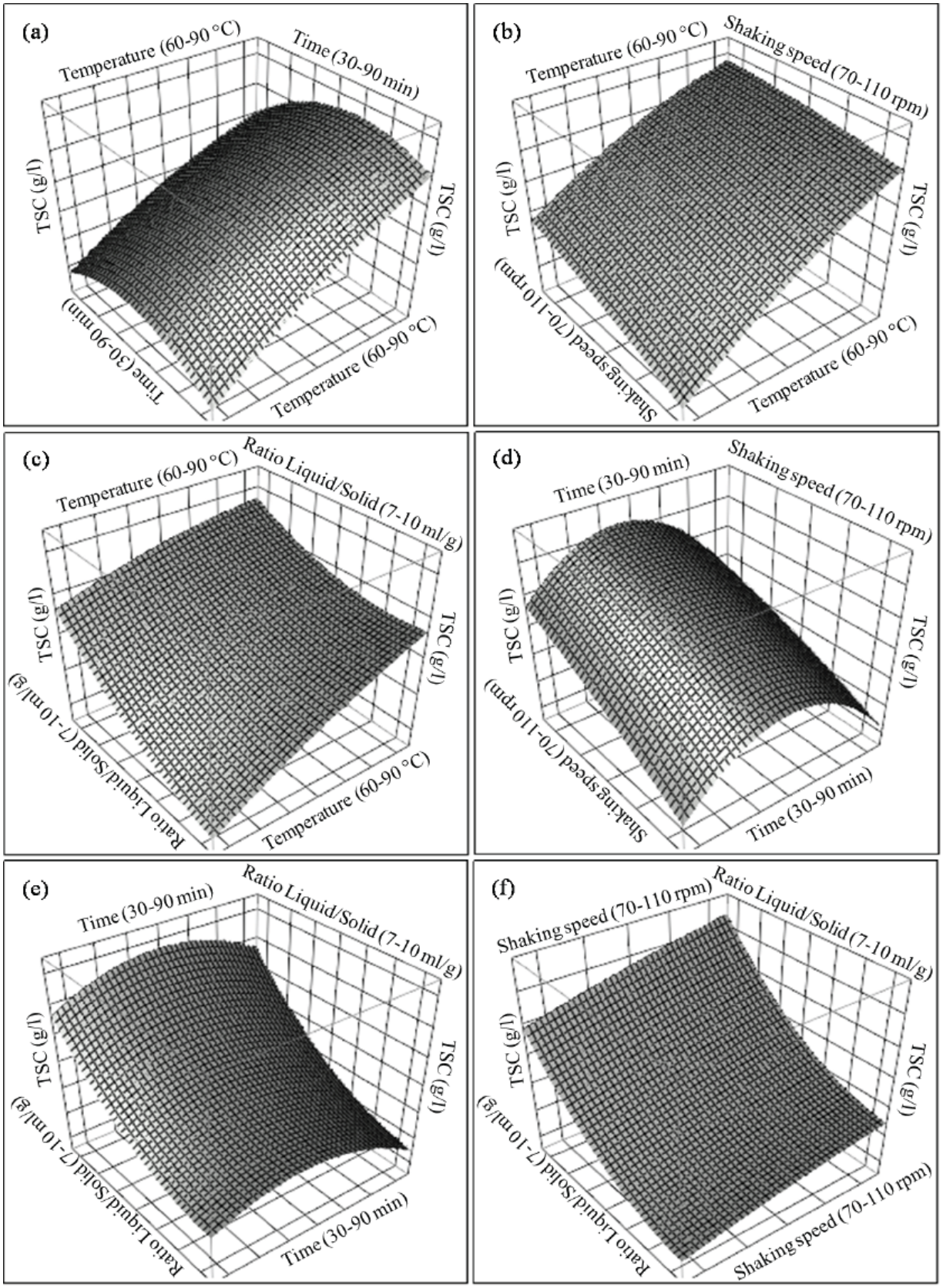

Fig. (3). Response surface analysis for the total sugar yield from *Phoenix dactylifera* L. by water bath extraction with respect to temperature and extraction time (**a**); temperature and shaking level (**b**); temperature and liquid/solid ratio (**c**); extraction time and shaking level (**d**); extraction time and liquid/solid ratio (**e**); shaking level and liquid/solid ratio (**f**).

3.5. Fitting the Response Surface Models

The empirical relationship between the extraction yield of TSC and the independent variables for MAE, UAE and CE, respectively, expressed by the quadratic model, are given in Eqs. (3), (4) and (5).

$$Y(TSC) = 222.59 + 3.16X_1 - 0.79X_2 + 18.52X_3 + 1.51X_1X_2 + 1.73X_1X_3 + 0.49X_2X_3 - 16.53X_1^2 - 2.39X_2^2 - 8.23X_3^2 \quad (3)$$

$$Y(TSC) = 179.42 + 10.03X_1 - 1.06X_2 + 15.11X_3 + 0.30X_1X_2 - 2.28X_1X_3 + 1.94X_2X_3 + 2.87X_1^2 - 2.23X_2^2 - 2.41X_3^2 \quad (4)$$

$$Y(TSC) = 201.52 + 17.03X_1 - 1.66X_2 + 5.38X_3 + 16.06X_4 - 2.04X_1X_2 - 2.35X_1X_3 - 0.65X_2X_3 - 5.77X_1X_4 + 1.42X_2X_4 + 0.74X_3X_4 - 4.18X_1^2 - 9.40X_2^2 - 0.85X_3^2 + 5.73X_4^2 \quad (5)$$

where Y is the extraction yield of TSC and X_1, X_2, X_3 and X_4 are the uncoded variables.

In general, the exploration and optimization of a fitted response surface may produce poor or misleading results unless the model exhibits a good fit, which makes it essential to verify the model's adequacy [30-32]. Table **3** shows the results of the second-order response surface in the form of analysis of variance (ANOVA). The significance of each coefficient was determined by the *F*-test and *p*-value, which are listed in Table **3**. The corresponding variables would be more significant if the absolute *F*-value becomes greater and the *p*-value becomes smaller [33].

It can be seen that the independent variables, microwave power (X_1), irradiation time (X_2) and liquid/solid ratio (X_3) for MAE, ultrasonic temperature (X_1), ultrasonic treatment time (X_2) and liquid/solid ratio (X_3) for UAE and for WBE, temperature (X_1), treatment time (X_2), liquid/solid ratio (X_3) and shaking speed (X_4), with the largest effect on the response ($p<0.0001$) were the linear terms. The results suggest that the change in these variables have highly significant effects on the yield of TSC ($p<0.0001$), when MAE, UAE and CE are used to extract sugars from *Phoenix dactylifera* L. The non-significant value of the lack of fit (more than 0.05) showed that the quadratic models were valid for the present study.

The Fisher's *F*-values (4040.3), (2751.5) and (8151.5) with a very low probability value [($p>F$) < 0.0001] implied that the models were highly significant. The goodness of fit of the models were examined by determination of coefficients (R_1^2=0.9940), (R_2^2=0.9910) and (R_3^2=0.9930) for MAE, UAE and CE, respectively, which implied that the samples variation (99.40,99.10 and99.30%) were statistically significant and only 0.06 %, 0.09 % and 0.07 %, respectively, of the total variance could not be explained by the model. The adjusted determination coefficients (Adj. R_1^2=0.9831) (Adj. R_2^2=0.9747) (Adj. R_3^2=0.9849) and the predicted determination coefficients (Pred. R_1^2=0.8881) (Pred. R_2^2=0.9448) (Pred. R_3^2=0.9035) were also satisfactory to confirm the significance of the models.

3.6. Comparative Study of the Three Extraction Processes (MAE, UAE and CE)

Date syrups were made from date juices extracted by three methods. The two alternative extraction technologies, MAE and UAE, were compared with each other and with CE considering the TSC yield of the extracts. A set of samples were extracted under the optimum conditions, and the predicted results fitted well with the experimental results. The following results were obtained: 233.80 ± 1.90g/l for MAE, 202.04 ± 3.40g/l for UAE and 234.38 ± 2.43 g/l for CE.

The selection of an extraction method would mainly depend on the advantages and disadvantages of the processes such as extraction yield, complexity, production cost, environmental friendliness and safety [34]. The results indicated that MAE showed a similar extraction capacity for the TSC as compared to CE; however, the extracted TSC by UAE was significantly lower than that of MAE and CE. This result is not in agreement with that reported by Ganbi (2012) [35], who found the highest total sugar content (88.94%) in the syrup extracted by ultrasonic procedure. The concentration using MAE greatly reduced the processing time; the shortest processing time (1.99 min) occurred at the power of 530 W. However, conventional extraction took the same time as ultrasound-assisted extraction, which was about 60 min. Microwave-assisted extraction was the most efficient method for extracting sugars based on the lower extraction time.

3.7. Identification of Sugars from Date Syrups by HPLC

Carbohydrates are the major chemical constituents of dates, including mainly reducing sugars such as glucose and fructose, and also non-reducing sugars such as sucrose, and small amounts of polysaccharides such as cellulose and starch [36]. In the present study, reducing sugars (glucose and fructose) and sucrose were identified in the date syrups obtained by MAE, UAE and CE methods (39.89, 16.64 and 23.25 %; 00.00, 46.94 and 53.06% and 49.22, 20.67 and 28.55 %, respectively). Concerning these two reducing sugars, all syrups contain more fructose than glucose, which is in agreement with previous studies [37]. High-fructose syrup is largely used as a sweetener in the food and pharmaceutical industries, as well as to attain crystalline fructose. Moreover, compared to sucrose syrup, it has more desirable functional properties, such as high osmotic pressure, high solubility, and itprevents the crystallization of sugar in food products [38]. Glucose and fructose were the only monomers of the reducing sugars present in date syrup, and they are the most important sources of energy with high digestibility [39]. These two sugars are the product of the enzymatic reaction of invertase on the disaccharide (sucrose). This enzyme hydrolyses sucrose into glucose and fructose completely in soft date cultivars, and partially in semi-dry and dry date cultivars [35]. Indeed, the increase in invertase activity is the main factor for the reducing sugar increase [40], and a direct correlation was found between the reducing sugar content of dates and the invertase activities [38]. As we can see, and

Table 3. Analysis of variance (ANOVA) for the experimental results of MA, UA and WB extractions.

MAE				UAE				WBE			
	Sum of Squares	**F-value**	**P-value**		**Sum of Squares**	**F-value**	**P-value**		**Sum of Squares**	***F*-value**	***p*-value**
Model	4040.3	91.4	< 0.0001	Model	2751.5	60.89	< 0.0001	Model	8151.5	122	< 0.0001
X_1-Power	3.16	4.04	0.0099	X_1-Tem-perature	10.03	12.66	< 0.0001	X_1- Tem-perature	17.03	26.9	< 0.0001
X_2-Time	-0.79	-1.00	0.3613	X_2-Time	-1.06	-1.43	0.2368	X_2-Time	-1.66	-2.63	0.0221
X_3-Ratio	18.52	23.63	< 0.0001	X_3-Ratio	15.11	19.07	< 0.0001	X_3-Shaking	5.38	8.53	< 0.0001
X_1X_2	1.51	1.36	0.2305	X_1X_2	0.31	0.28	0.79	X_4-Ratio	16.06	25.5	< 0.0001
X_1X_3	1.72	1.56	0.1795	X_1X_3	-2.28	-2.04	0.10	X_1X_2	-2.04	-1.86	0.0869
X_2X_3	0.49	0.45	0.6744	X_2X_3	1.94	1.74	0.14	X_1X_3	-2.35	-2.15	0.0529
X_1^2	-16.53	-14.33	< 0.0001	X_1^2	2.87	2.46	0.06	X_1X_4	-5.77	-0.59	0.5641
X_2^2	-2.39	-2.07	0.0928	X_2^2	-2.23	-1.91	0.11	X_2X_3	-0.65	-5.28	0.0002
X_3^2	-8.23	-7.13	0.0008	X_3^2	-2.41	-2.07	0.09	X_2X_4	1.42	1.30	0.2183
Residual	24.56			Residual	25.11			X_3X_4	0.74	0.68	0.5106
Lack of Fit	91.40	1.30	0.46	Lack of Fit	60.89	1.07	0.52	X_1^2	-4.18	-4.42	0.0008
Pure Error	8.32			Pure Error	9.64			X_2^2	-9.40	-9.93	< 0.0001
R^2	0.99			R^2	0.99			X_3^2	-0.85	-0.90	0.0008
R^2_{adj}	0.98			R^2_{adj}	0.97			X_4^2	5.73	6.05	< 0.0001
Adeq pre				Adeq pre				Residual	57.29		
RMSE	2.22			RMSE	2.24			Lack of Fit	121.95	0.40	0.87
Cor Total	24.56			Cor Total	25.11			Pure Error	19.03		
								R^2	0.99		
								R^2_{adj}	0.98		
								Adeq pre			
								RMSE	2.19		
								Cor Total	8208.8		

except for the syrup obtained by UAE, the major sugar is the non-reducing one (sucrose) in both MAE and CE syrups; theircontent represents more than half (60.11 %) of the reducing sugars in the syrup obtained by MAE and about half (50.78 %) of that prepared by CE or WBE. Indeed, dry dates contain relatively more sucrose than reducing sugars [41], which confers a very pleasant taste to the fruit [42]. It is also worth highlighting the absence of sucrose in the syrup obtained by UAE, which could be explained by the increases of invertase activity, during the ultrasound treatment. It has been reported that sucrose hydrolysis by invertase is accelerated by ultrasound irradiation [43]. On the other hand, the reducing sugar release, in cassava chip slurry, was as high as 180% of the control samples and the slurry samples with enzyme addition during sonication resulted in greater release than samples with enzyme addition after sonication [44].

CONCLUSION

In this study, novel extraction techniques (MAE and UAE) were compared with a conventional one in the extraction of TSC from *Phoenix dactylifera* L. and RSM was suc-

cessfully applied to optimize the three extraction methods. Microwave power, ultrasonic temperature, water bath temperature, extraction time, liquid/solid ratio and shaking speed played significant roles in the extraction of the investigated compounds. We were able to extract higher yields of the constituent from *Phoenix dactylifera* L., while using microwave-assisted extraction, which simultaneously reduced the extraction time considerably compared with two other methods that take along extraction time. However, if we consider the nature of the sugars extracted by these three methods, the ultrasound technique is the one that gives the best yields of reducing sugars and especially fructose, which is known for its desirable functional properties.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No Animals/Humans were used for studies that are the basis of this research.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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Declared none.

REFERENCES

[1] Aspé E, Fernández K. The effect of different extraction techniques on extraction yield, total phenolic, and anti-radical capacity of extracts from *Pinus radiata* Bark. Ind Crops Prod 2011; 34: 838-44.

[2] Dahmoune F, Boulekbache L, Moussi K, Aoun O, Spigno G, Madani K. Valorization of Citrus limon residues for the recovery of antioxidants: Evaluation and optimization of microwave and ultrasound application to solvent extraction. Ind Crops Prod 2013; 50: 77-87.

[3] Aybastıer Ö, Işık E, Şahin S, Demir C. Optimization of ultrasonic-assisted extraction of antioxidant compounds from blackberry leaves using response surface methodology. Ind Crops Prod 2013; 44: 558-65.

[4] Muniz-Marquez DB, Martinez-Avila GC, Wong-Paz JE, Belmares-Cerda R, Rodriguez-Herrera R, Aguilar CN. Ultrasound-assisted extraction of phenolic compounds from Laurus nobilis L. and their antioxidant activity. Ultrason Sonochem 2013; 20: 1149-54.

[5] Chandrasekaran M, Bahkali AH. Valorization of date palm (*Phoenix dactylifera*) fruit processing by-products and wastes using bioprocess technology - Review. Saudi J Biol Sci 2013; 20: 105-20.

[6] Abbès F, Kchaou W, Blecker C, Ongena M, Lognay G, Attia H, Besbes S. Effect of processing conditions on phenolic compounds and antioxidant properties of date syrup. Ind Crops Prod 2013; 44: 634-42.

[7] Besbes S, Drira L, Blecker C, Deroanne C, Attia H. Adding value to hard date (*Phoenix dactylifera* L.): Compositional, functional and sensory characteristics of date jam. Food Chem 2009; 112: 406-11.

[8] Abbès F, Bouaziz MA, Blecker C, Masmoudi M, Attia H, Besbes S. Date syrup: Effect of hydrolytic enzymes (pectinase/cellulase) on physico-chemical characteristics, sensory and functional properties. LWT - Food Sci Technol 2011; 44: 1827-34.

[9] Boudries H, Kefalas P, Horneromendez D. Carotenoid composition of Algerian date varieties (*Phoenix dactylifera*) at different edible maturation stages. Food Chem 2007; 101: 1372-7.

[10] Baliga MS, Baliga BRV, Kandathil SM, Bhat HP, Vayalil PK. A review of the chemistry and pharmacology of the date fruits (*Phoenix dactylifera* L.). Food Res Int 2011; 44: 1812-22.

[11] Al-Hooti SN, Sidhu JS, Al-Saqer JM, Al-Othman A. Chemical composition and quality of date syrup as affected by pectinase/cellulase enzyme treatment. Food Chem 2002; 79: 215-20.

[12] Gad A, Kholif A, Sayed A. Combination of date palm syrup and skim milk. Am J Food Technol 2010; 5: 250-59.

[13] Manaa S, Younsi M, Moummi N. Study of methods for drying dates; Review the traditional drying methods in the region of Touat Wilaya of Adrar-Algeria. Energy Procedia 2013; 36: 521-4.

[14] Benmeddour Z, Mehinagic E, Meurlay DL, Louaileche H. Phenolic composition and antioxidant capacities of ten Algerian date (*Phoenix dactylifera* L.) cultivars: A comparative study. J Funct Foods 2013; 5: 346-54.

[15] Chibane H, Benamara S, Noui Y, Djouab A. Some physicochemical and morphological characterizations of three varieties of Algerian common dates. Eur J Sci Res 2007; 18: 134-40.

[16] El Ogaïdi H. Dates and confectionery product. FAO Rome 1987; 1-25.

[17] Munier P. Le palmier-dattier, Techniques agricoles et productions tropicales. Maisonneuve & Larose: Paris 1973.

[18] Guérin C. Première biozonation du Pléistocène européen, principal résultat biostratigraphique de l'étude des Rhinocerotidae (Mammalia, Perissodactyla) du Miocène terminal au Pléistocène supérieur d'Europe occidentale. Geobios 1982; 15: 593-8.

[19] Hashemi SMB, Khaneghah AM, Saraiva JA, Jambrak AR, Barba FJ, Mota MJ. Effect of ultrasound on lactic acid production by *Lactobacillus* strains in date (*Phoenix dactylifera var. Kabkab*) syrup. Appl Microbiol Biotechnol 2018; 102:2635-44.

[20] Multon J. interet des additifs pour ameliorer la conservation des aliments, Additifs et auxiliaires de fabrication dans les industries agro-alimentaires/coordonnateur, JL Multon; preface, F. Lepatre 1984.

[21] Dreywood R. Qualitative test for carbohydrate material. Ind Eng Chem Anal Ed 1946; 18: 499.

[22] Hanson R, Phillips J. Chemical composition. Manual of methods for general bacteriology. American Society for Microbiology: Washington DC 1981; pp. 328-64.

[23] Sahin S, Samli R. Optimization of olive leaf extract obtained by ultrasound-assisted extraction with response surface methodology. Ultrason Sonochem 2013; 20: 595-602.

[24] Sun Y, Wang W. Ultrasonic extraction of ferulic acid from Ligusticum chuanxiong. J Chin Inst Chem Eng 2008; 39: 653-6.

[25] Zou Y, Chen X, Yang W, Liu S. Response surface methodology for optimization of the ultrasonic extraction of polysaccharides from *Codonopsis pilosula* Nannf. var. *modesta* LT Shen. Carbohydr Polym 2011; 84: 503-8.

[26] Ji J, Lu X, Cai M, Xu Z. Improvement of leaching process of geniposide with ultrasound. Ultrason sonochem 2006; 13: 455-62.

[27] Vilkhu K, Mawson R, Simons L, Bates D. Applications and opportunities for ultrasound assisted extraction in the food industry—A review. Innov Food Sci Emerg Technol 2008; 9: 161-9.

[28] Han D, Zhu T, Row KH. Ultrasonic extraction of phenolic compounds from *Laminaria japonica* Aresch using ionic liquid as extraction solvent. Bull Korean Chem Soc 2011; 32: 2212-6.

[29] Fernandez D, Goodwin A, Lemmon EW, Sengers JL, Williams R. A formulation for the static permittivity of water and steam at temperatures from 238 K to 873 K at pressures up to 1200 MPa, including derivatives and Debye-Hückel coefficients. J Phys Chem Ref Data 1997; 26: 1125-66.

[30] Bimakr M, Rahman RA, Ganjloo A, Taip FS, Salleh LM, Sarker MZI. Optimization of supercritical carbon dioxide extraction of bioactive flavonoid compounds from spearmint (*Mentha spicata* L.) leaves by using response surface methodology. Food Bioproc Tech 2012; 5: 912-20.

[31] Liyana-Pathirana Cand Shahidi F. Optimization of extraction of phenolic compounds from wheat using response surface methodology. Food Chem 2005; 93: 47-56.

[32] Scavroni J, Boaro CSF, MarquesMOM, Ferreira LC. Yield and composition of the essential oil of *Mentha piperita* L.(Lamiaceae) grown with biosolid. Braz J Plant Physiol 2005; 17: 345-52.

[33] Atkinson A, Donev A. Optimum experimental designs. Clarendon Press: UK 1992.

[34] Li H, Deng Z, Wu T, Liu R, Loewen S, Tsao R. Microwave-assisted extraction of phenolics with maximal antioxidant activities in tomatoes. Food Chem 2012; 130: 928-36.

[35] Ganbi HHA. Production of nutritious high quality date (*Phoenix dactylifera*) fruits syrup (dibs) by using some novel technological approaches. J Appl Sci Res 2012; 8: 1524-38.

[36] Ashraf Z, Hamidi-Esfahani Z. Date and date processing: A review. Food RevInt2011; 27: 101-33.

[37] Al-Eid SM. Chromatographic separation of fructose from date syrup. Int J Food Sci Nutr 2006; 57: 83-96.

[38] Chaira N, Smaali I, Besbes S, Mrabet A, Lachiheb B, Ferchichi A. Production of fructose rich syrups using invertase from date palm fruits. J Food Biochem2011; 35: 1576-82.

[39] Al-Farsi M, Alasalvar C, Al-Abid M, Al-Shoaily K, Al-Amry M, Al-Rawahy F. Compositional and functional characteristics of dates, syrups, and their by-products. Food Chem 2007; 104: 943-7.

[40] Hasegawa S, Smolensky DC. Date invertase: Properties and activity associated with maturation and quality. J Agr Food Chem 1970; 18: 902-4.

[41] Eltayeb EA, Al-hasni AS, Farooq SA. Changes in soluble sugar content during the development of fruits in some varieties of omani date palm (*Phoenix dactylifera*). Pak J Biol Sci 1999; 2: 255-8.

[42] Mrabet A, Ferchichi A, Chaira N, Mohamed BS. Physico-chemical characteristics and total quality of date palm varieties grown in the Southern of Tunisia. Pak J Biol Sci 2008; 11: 1003-8.

[43] Kwiatkowska B, Bennett J, Akunna J, Walker GM, Bremner DH. Stimulation of bioprocesses by ultrasound. Biotechnol Adv 2011; 29: 768-80.

[44] Nitayavardhana S, Rakshit SK, Grewell D. Ultrasound pretreatment of cassava chip slurry to enhance sugar release for subsequent ethanol production. Biotechnol Bioengin 2008; 101: 487-96.