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Optimization of date seed oil extraction using the assistance of hydrothermal and ultrasound technologies

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SUMMARY: The date seed is a by-product from the date industry. Its use as a source of added-value compounds is of great interest. Oil accounts for 5-13% of the seed's weight. Soxhlet extraction with organic solvents is the traditional method for obtaining oil from seeds. In this work, hydrothermal pre-treatments and sonication are proposed to make the extraction a more environmentally friendly process. Factors such as sonication time and temperature and hexane-to-seed ratio (H/S) have been considered. Response surface methodology was applied for optimization. Hydrothermal treatments increased oil recovery. H/S was the most influential factor, and was close to 7 mL/g seeds for both samples. 71% recovery was achieved for native seeds after 15 min sonication at 45 ºC, and 80% for 180 ºC-treated seeds after 45 min at 35 ºC when compared to Soxhlet extraction. These conditions comply with our initial aim. Pre-treatments seem to have a negative effect on oil stability, although this observation needs to be confirmed.

KEYWORDS: *Date seed; Hydrothermal technology; Oil extraction; Oxidation stability; Response surface methodology; Sonication*

RESUMEN: ***Optimización de la extracción de aceite de semilla de dátil mediante la ayuda de tecnologías hidrotermales y de ultrasonido.*** La semilla de dátil es un subproducto de la industria datilera. Su uso como fuente de compuestos de valor añadido sería muy interesante. El aceite representa el 5-13% del peso de la semilla. La extracción mediante Soxhlet con disolventes orgánicos es el método tradicional para obtener aceite de semillas. En este trabajo se proponen pretratamientos hidrotérmicos y sonicación para diseñar un proceso de extracción más respetuoso con el medio ambiente. Se han considerado factores como el tiempo y la temperatura de sonicación y la proporción hexano/semilla (H/S). La optimización del proceso se llevó a cabo por el método de superficie de respuesta. Los tratamientos hidrotérmicos aumentaron el porcentaje de recuperación. El factor más influyente fue H/S, y su valor óptimo estuvo cerca de 7 mL/g para ambas muestras. La recuperación óptima fue del 71% para las semillas sin tratamiento tras 15 min de sonicación a 45 ºC, y del 80% para las semillas tratadas a 180 ºC después de 45 min a 35 ºC. Estos resultados cumplen con nuestro objetivo inicial. Sin embargo, los pretratamientos parecen tener un efecto negativo sobre la estabilidad del aceite, lo que se confirmará en estudios posteriores.

PALABRAS CLAVE: *Estabilidad oxidativa; Extracción de aceite; Método de superficie de respuesta; Semilla de dátil; Sonicación; Tratamientos hidrotérmicos*

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1. INTRODUCTION

The date palm has long been one of the most important fruit crops and it is widely distributed in many regions of the world, including Middle Eastern countries and North Africa (El-Rahman and Al-Mulhem, 2017; Djaoudene *et al.*, 2019). The date palm is a multipurpose tree with nutritional, therapeutic, and environmental attributes (Daoud *et al.*, 2015), and plays an important role at economic and social levels for people from arid and semiarid regions of the world (Besbes *et al.*, 2004). At present, its production and consumption are continuously increasing: in 2018, more than 8.5 million tons of dates were produced, with an annual by-product yield of approximately 2.5 million tons of date seeds from date fruit processing industries (pitted dates, date powders, date syrup, date juice, chocolate-coated dates and date confectionery) (Basuny and Al-Marzooq, 2011). The fruit from the date palm is composed of a fleshy pericarp and a seed, which constitutes 10 to 15% of the date's weight. Although the date seed is a good source of cattle food, Kchaou *et al.* (2013) reported that the disuse of this by-product represents a real economic loss since it offers a promising raw material for the extraction of bioactive compounds. In addition to dietary fiber, proteins and minerals, date seeds present a content in oil between 5-13% on dry weight basis, which is rich in phenolics, tocopherols and phytosterols (Besbes *et al.*, 2004; Mrabet *et al.*, 2015). The composition of date seed oil has been studied by other authors, and its content in antioxidants, vitamins, minerals and fatty acids (50% oleic acid, 19% linoleic acid, 10% lauric acid, and 10% palmitic acid) makes it valuable for food formulation and cosmetic and pharmaceutical applications (Al Ghezi *et al.*, 2020; Mrabet *et al.*, 2020).

Extraction is the most important step in the recovery of this bioactive oil. In comparison to other seeds, the process for date seeds is hindered by the hardness of the raw material. Therefore, the application of a pre-treatment would be useful. A wide range of pre-treatment techniques for date seed oil extraction has already been reported (Kareem *et al.*, 2021), but they are all chemical-consuming processes. One of the most interesting environmental-friendly processes is based on hydrothermal pre-treatments which enable the extraction of soluble compounds into the liquid phase, leaving an oil-enriched fibrous material as a solid fraction (Fernández-Bolaños *et al.*, 2004). This technology has been successfully applied to several agricultural by-products, such as sugarcane bagasse (Boussarsar *et al.*, 2009) or corn cobs (Egües *et al.*, 2012) to obtain sugars, oligosaccharides, or phenols. Our research team has designed a 100-L reactor for the treatment of lignocellulosic material (Lama-Muñoz *et al.*, 2011) which works at mid pressures (up to 9 Kg/cm2) and can be applied to a wide range of agricultural by-products (Mrabet *et al.*, 2015; Rubio-Senent *et al.*, 2015; Fuentes-Alventosa *et al.*, 2013) without the addition of chemicals.

The most widely used procedures for oil extraction from a solid plant matrix are cold extraction and conventional Soxhlet extraction. However, these methods use large amounts of organic solvents which are released into the atmosphere and are very time- and energy-consuming (Wang and Weller, 2006). Nowadays, there is a need to use new technologies that can reduce extraction time and solvent consumption. Ultrasound-assisted extraction is based on the energy that ultrasound waves bring to an extraction medium and improves the mass transfer phenomena by cavitation, as well as promoting biomass diffusion, cell disruption, and solvent penetration (Barba *et al.*, 2016; Kaufmann and Christen, 2002). Several process variables such as extraction solvent, time, temperature, and liquid-to-solid ratio have great impacts on process optimization. Therefore, the different interactions among these factors could have significant effects on extraction yields. The optimization of oil extraction conditions is commonly achieved by statistical and mathematical approaches, usually by the use of response surface methodology (Bassani *et al.*, 2014; Sharif *et al.*, 2014). This statistical implement has been successfully used for optimizing the oil extraction conditions from avocado pulp (Xuan *et al.*, 2017), papaya seeds (Samaram *et al.*, 2015), winter melon seeds (Bimakr *et al.*, 2012) or capper seeds (Ara *et al.*, 2014).

The main objective of this work is to study the oil extraction from date seeds by the application of two environmentally friendly technologies (hydrothermal pre-treatments for conditioning the raw material and sonication for improving the oil yield) in comparison to traditional Soxhlet extraction. We studied several parameters, such as liquid-to-solid ratio, sonication time, and sonication temperature, and maximized the extraction process using response surface methodology. In addition, the oxidative stability of the optimized oils was presented as an initial assessment of oil quality.

2. MATERIALS AND METHODS

2.1. Sample preparation

Date seeds were collected randomly from the Nefzaoua area, located in the South West of Tunisia. The selected seeds were soaked in water, and then washed to remove any adhered date flesh and air dried. They were further oven-dried at 60 ºC for 24 h. The dried seeds were milled and sieved using a 1-mm mesh sieve and stored at -20 ºC until use.

2.2. Hydrothermal treatments

Steam treatments were carried out using a 100-L capacity reactor that can work at temperatures between 50 and 190 ºC by direct heating with saturated steam, and at a maximum pressure of 9 kg/cm^2. Date seed samples (10 Kg) were treated at temperatures between 140 and 190 ºC, as preliminary experiments. After treatment, the samples were collected, vacuum filtered through filter paper, dried, milled and sieved using a 1-mm mesh sieve and stored at -20 ºC.

2.3. Conventional soxhlet extraction

Date seed oil extraction was carried out on a solvent extractor equipped with six Soxhlet posts. 10 g of date seed powder were added to a cellulose thimble and placed into a Soxhlet apparatus that was connected to a 250-mL round-bottom flask containing 200 mL hexane. The extraction process lasted for 8 h and until the solvent in the reflux pipe became colorless. The extract was evaporated in a rotary vacuum evaporator (model R-210, Buchi, Flawil, Switzerland) at 40 °C, after which the collected oil was stored in vials at -20 °C until further analysis.

2.4. Ultrasound-assisted extraction (UAE)

The date seed powder was put into a screw-cap flask (250 mL), made up to required volume with hexane, and sonicated in an ultrasonic bath (Thermo-10D; 40 kHz frequency; 240 W ultrasonic output power; 500×300×150 mm internal dimensions). The seed and solvent mixture was irradiated at different levels of sonication time, temperature, and hexane-to-seed (H/S) ratio. The bath water was placed in an external thermostatic device to avoid auto-heating effects during sonication. Upon extraction completion, the extract was passed through filter paper in a funnel and the solvent was removed using a rotary vacuum evaporator (model R-210, Buchi, Flawil, Switzerland) at 40 °C. The extracted oils were stored at -20 °C until further analysis.

2.5. Experimental design

Response surface methodology with a three-factor, three-level Box–Behnken design was employed to study the effect of UAE parameters on the recovery of date seed oil. Three independent factors, namely sonication time (X_1, min), sonication temperature (X_2, °C), and H/S ratio (X_3, mL/g) were used. Three levels were applied to each factor (Table 1). The ranges of the variables studied were chosen according to the preliminary experiments. The response was oil recovery (%) referred to Soxhlet extraction yield. The experimental design and statistical analysis were created and performed using the Statgraphics Plus Version 2.1 software (Statgraphics Technologies Inc., The Plains, Virginia). The experimental design was composed of 15 experimental runs. The three replicates of the center points evaluated the repeatability of the designed method and were used to examine experimental error. A second-order polynomial model was used to fit the experimental data.

TABLE 1. Levels and values of independent variables

Variables	Symbol code	Levels		
		-1	0	1
Sonication temperature (ºC)	X_1	25	35	45
Sonication time (min)	X_2	15	30	45
Hexane (mL)/seed(g) ratio	X_3	3	5	7

The experiments were randomly conducted in order to avoid the effects of unexpected variability in the observed responses which resulted from extraneous factors. Analysis of variance (ANOVA) was used to analyze the individual linear, quadratic and interaction regression coefficients. The values of R^2 (coefficient of determination) and F-test were used to examine the adequacy of the designed model. Regression analysis and response surface plot were used to examine the optimal conditions and the effects of the interactions among independent variables. The confidence level was set at 95%. The validity of the designed model was confirmed by performing triplicate analyses on the generated optimal parameters and one-sample t-test was used to verify the differences between experimental and predicted values.

2.6. Oil oxidative stability

The oxidative stability of the oil (OOS) was evaluated by applying a modification of the thiobarbituric acid (TBA) reactive species method (Rodríguez *et al*., 2007). The degradation compounds (aldehydes, ketones, etc.) synthesized during oil oxidation reacted with TBA to give a pink-colored complex whose concentration was measured at 540 nm. Sixty microliters of oil and 5 µL of ABAP were added to an Eppendorf tube (1.5 mL capacity) and made up to 0.1 mL with distilled water (in quadruplicate). Afterwards, 150 µL 20% acetic acid (pH 3.5) and 150 µL 0.8% w/v TBA in 1.1% sodium dodecylsulphate w/v were measured into each tube. This mixture was stirred in a Vortex and heated at 80 ºC for 1 h. After cooling at room temperature, 0.5 mL of 1-butanol were added, stirred and centrifuged at 12,100 g for 3 min. The absorbance of the butanol layer was measured at 540 nm. High absorbance values indicated high concentrations of TBA-reactive species quantified in the reaction medium and therefore low oxidative stability in the assayed oil. As the lower the absorbance the higher the stability, for reasons of clarity the final oil oxidative stability (OOS) was expressed as:

$$OOS = 1/Abs_{540}$$

2.7. Statistical analysis

To assess the differences among samples, a multiple sample comparison was performed using the Statgraphics® Plus program Version 2.1. A multifactorial analysis of variance (ANOVA), followed by Duncan's multiple comparison test was performed to differentiate the samples. The level of significance was $p < 0.05$.

3. RESULTS AND DISCUSSION

The ultrasound technique was studied as assistance for a less contaminating and less energy-consuming hexane extraction compared to the traditional Soxhlet extraction. In addition, hydrothermal pre-treatments were applied to the date seeds in order to disorganize their structure and to ease the oil release from the seed tissue. Statistical designs were developed to study the significance of each parameter (sonication temperature and time, H/S ratio, and the implementation of a pre-treatment). Multifactorial ANOVA and response surface models were applied for identifying significant factors, formulating regression equations and finding the optimized conditions for each experiment.

3.1. Oil yield of the native and pre-treated date seeds

The control and hydrothermally-treated samples were subjected to Soxhlet extraction and the obtained yields were considered as reference values for the ultrasound-assisted experiments. After preliminary experiences, the highest oil yield by the Soxhlet method was obtained at 180 ºC. This fact suggests a very intense disorganization of seed tissues which enabled the extraction of higher amounts of oil, together with the enrichment in oil of the solid recovered after pre-treatment (Fernández-Bolaños *et al*., 2004) due to the partial solubilization of the solid material into the treatment liquid. In a previous work (Mrabet *et al*., 2015), cellulose, lignin and mannans coming from date seeds were quantified in the solid residue recovered after hydrothermal treatments of complete date fruits, pointing to a partial degradation of seed structure. This disorganization of lignocellulosic material (the solubilization of lignin and hemicelluloses and the enrichment with cellulose and oil of the resultant solid) have been described when working with other agricultural by-products (Rubio-Senent *et al*., 2015; Fuentes-Alventosa *et al*., 2013). For the native date seed, a lower oil yield was obtained (7.67 ± 0.06%) compared to pre-treated ones (Besbes *et al*., 2004) or 11.8-15.6% (Mrabet *et al*., 2015) for some Tunisian varieties, and the lowest for some Algerian ones (5.4-5.6%) (Boukouada *et al*., 2014). Surface response methodology was applied to samples, both the native seeds and 180 ºC-treated ones, for oil recovery optimization.

3.2. Oil recovery for the different experiments

The different parameters and levels summarized in Table 1 were applied to both samples (native date seeds and with hydrothermal pretreatments at 180 ºC), and two different Box-Behnken designs were developed (Tables 2 and 3). Each model was composed of fifteen experiments plus three at optimal conditions to validate the designed model. The oil yield was the response variable studied because it is the main parameter considered to study the economic viability of the extraction process. The obtained oil recovery by UAE was between 42-73% and 57-81% of the oil extracted by Soxhlet for native seeds and those treated at 180 ºC, respectively. In spite of these yields, the savings in energy, solvent, and working time could make this system valuable. In addition, the chemical composition of UAE oils should be less modified when compared to the corresponding Soxhlet ones, probably due to a gentle exposure to temperature; but it is also important to keep in mind that ultrasound application could decrease the oxidative stability of oil (Samaram *et al*., 2015; Böger *et al*., 2018). The extra energy applied to plant materials by ultrasound is one of its advantages, because interesting biomolecules are extracted in higher concentrations, e.g. phenols and other antioxidants (Bimakr *et al*., 2012). However, this energy is also the activation energy for the appearance of free radicals, molecules that trigger oxidation chain reactions, which are responsible for the characteristic rancid color and flavor. These defects negatively affect the shelf-life and consumer acceptance of food products and are a major cause of food waste (Böger *et al*., 2018).

Table 2. Box-Behnken design with the different combinations of the three independent variables (sonication temperature X_1, sonication time X_2, and hexane/seed ratio X_3) for ultrasound-assisted hexane extraction optimization of natural date seeds.

Run order	Independent variables			Oil recovery (%)
	X_1	X_2	X_3	
1	25	30	7	73.27
2	25	15	5	61.93
3	25	30	3	48.89
4	35	30	5	55.41
5	35	30	5	55.41
6	35	15	7	65.19
7	45	30	7	70.01
8	45	45	5	60.23
9	45	15	5	61.93
10	35	45	7	65.19
11	25	45	5	61.93
12	35	30	5	55.41
13	35	15	3	45.63
14	45	30	3	50.46
15	35	45	3	42.37

Table 3. Box-Behnken design with the different combinations of the three independent variables (sonication temperature X_1, sonication time X_2, and hexane/seed ratio X_3) for ultrasound-assisted hexane extraction optimization of date seeds treated at 180 ºC.

Run order	Independent variables			Oil recovery (%)
	X_1	X_2	X_3	
1	25	30	7	73.73
2	35	30	5	75.30
3	35	45	3	64.70
4	45	15	5	66.27
5	35	45	7	81.33
6	25	30	3	63.25
7	45	30	3	64.70
8	35	15	7	75.30
9	45	45	5	78.31
10	45	30	7	73.73
11	35	15	3	57.23
12	35	30	5	72.29
13	35	30	5	72.29
14	25	45	5	72.29
15	25	15	5	70.72

The high energy of ultrasonic sound waves provokes the disruption of the cell wall, thus making the oil more accessible and more easily extracted (Vinatoru, 2001). During sonication, cycles of compression and decompression are originated in the liquid. During compression, little bubbles are generated in the solvent that lead to localized pressure increases. But during decompression, the bubbles implode, collapse and originate rarefactions. These cycles are also known as cavitation phenomenon, which is responsible for cell wall disruption, oil release into the solvent and destruction of oil emulsion (Lou *et al*., 2010). The date seed is a very hard by-product from date fruit processing with a chemical composition that suggests a very complex and closed cell wall structure: 20-25% lignin, 17-20% cellulose and 11-20% hemicelluloses, 60% of which are mannans (Mrabet *et al*., 2015). Mannans establish many closed complexes with cellulose microfibers, which tighten the cell wall network (Whitney *et al*., 1998). These facts could hinder oil extraction. Hydrothermal pre-treatments change the structure of the plant material, dissolving most of the lignin and loosening the lignocellulosic materials (Fernández-Bolaños *et al*., 2004; Jaramillo-Carmona *et al*., 2019). The added effects of both processes, ultrasound and hydrothermal treatments, could be the reason for the higher extraction yields and oil recoveries in the sample treated at 180 ºC.

A multifactorial ANOVA was applied to all these assays, and the application of a pre-treatment was included as a factor. The results are presented in Table 4. The sonication temperature was the only factor that did not show significance, although it effects were variable when working with other vegetal products, e.g. winter melon seeds (Bimakr *et al.*, 2012) and caper seed (Ara *et al.*, 2014). In the cited works, the yield increased with temperature until it reached its maximum. This fact could be related to an increased diffusivity of the solvent into cells and to an enhanced solubility of oil from the cells into solvent. At higher temperatures, the yield decreases were probably due to a decrease in the number of acoustic bubbles generated by sonication. H/S ratio and the pre-treatment had the greatest influence (Table 4). At a high H/S ratio, there was sufficient solvent capacity for oil transfer and enhanced diffusion through a viscosity reduction. Both circumstances led to increases in the concentration gradient and to a more efficient extraction (Samaran *et al.*, 2015). All these phenomena were eased by the disruption of the matrix structure caused by hydrothermal pre-treatments. The effect of the different significant factors is graphically displayed in Fig 1 as the recovery average values and LSD intervals.

Table 4. Multifactorial ANOVA results for oil recovery (%) and four factors (sonication temperature, sonication time, hexane/solid ratio, and pre-treatment).

Source	Sum of squares	DF	Mean square	F-ratio	p-value
Temperature	30.8169	2	15.4085	1.11	0.3489
Time	45.9242	2	22.9621	2.38	0.0467
H/S ratio	1266.38	2	633.19	45.41	0.0000
Pretreatment	1180.39	1	1180.39	84.65	0.0000
Residual	306.758	22	13.9435		
Cor. total	2836.76	29			

3.3. Statistical models for date seed oil recovery

The best-fitting models were generated by including only the significant terms ($p < 0.05$) in the generated quadratic models. Table 5 shows the results of the ANOVA analysis for the reduced quadratic model of the samples analyzed. These models can explain most of the variations in oil recovery, as shown by the high F values in the models (816.22 and 25.40 for native and 180 ºC-treated date seeds, respectively). This value decreased with pretreatment, and the same happened with the coefficient of determinations (R^2 and adjusted R^2). This suggested a lower fit for treated seeds than for the native ones. Nevertheless, the models displayed high adjusted R^2, with values > 0.80.

Table 5. Analysis of variance (ANOVA) for the response surface quadratic model of date seed oil recovery.

Source	Sum of squares	DF	Mean square	F-ratio	p-value
Native date seeds					
X_1	1.436	1	1.436	7.57	0.0284
X_2	3.075	1	3.075	16.21	0.0050
X_3	931.177	1	931.177	4908.31	0.0000
X_1^2	137.191	1	137.191	723.14	0.0000
X_1X_3	5.832	1	5.832	30.74	0.0009
X_2X_3	2.657	1	2.657	14.00	0.0072
X_3^2	2.574	1	2.574	13.57	0.0078
Pure error	1.328	7	0.190		
Cor. total	1088.66	14			
R^2 99.90; adjusted R^2 97.33					
180 ºC-treated date seeds					
X_2	91.858	1	91.858	10.16	0.0078
X_3	367.434	1	367.434	40.65	0.0000
Pure error	108.473	12	9.039		
Cor. total	567.765	14			
R^2 87.89; adjusted R^2 80.89					

For native date seeds, the H/S ratio (X_3) was the most influential factor (Table 5), followed by the second-order term of sonication temperature (X_1^2) and interaction term between temperature and H/S ratio (X_1X_3). In addition, sonication time (X_2), interaction term between time and H/S ratio (X_2X_3), second-order term of H/S ratio (X_3^2), and sonication temperature (X_1) were significant. The significant interactions among variables are presented in Figure 2 as response surfaces. The interaction between temperature and H/S ratio (X_1X_3) (Sub-Figure 2.1) showed that at low and high temperatures the recovery increased, especially at a high H/S ratio. The interaction between time and H/S ratio (X_2X_3) (Sub-Figure 2.2) was less significant, and the recovery decreased at a low H/S ratio and high sonication time, showing the opposite effect at a

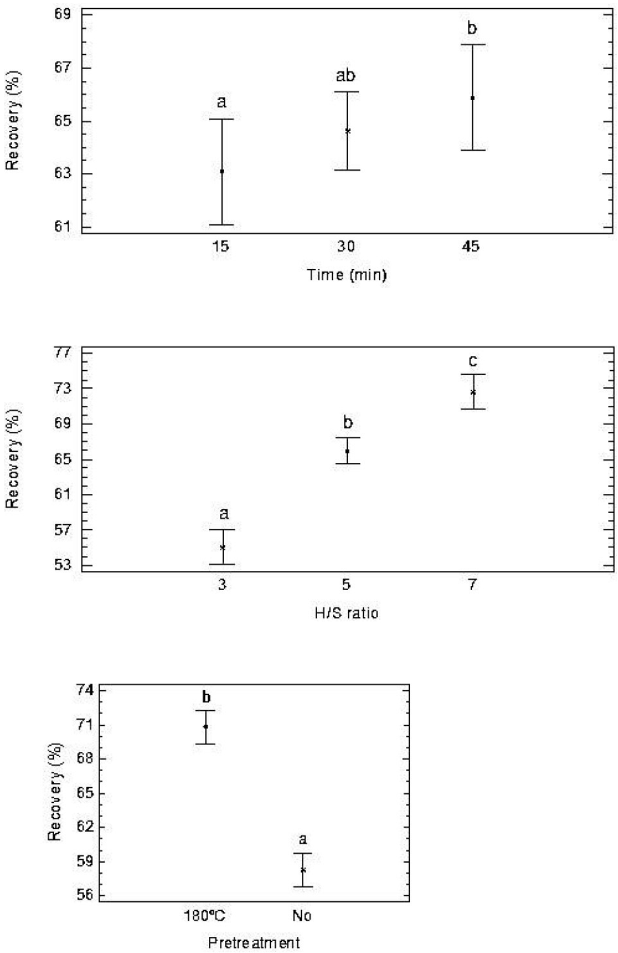

FIGURE 1. ANOVA average values for oil recovery and LSD intervals for the different significant factors: A, sonication time; B, H/S ratio; C, pretreatment.

high H/S ratio. The reduced quadratic model that shows the relationship between the different factors and oil recovery is:

$$Y = 93.927 - 3.995X_1 - 0.177X_2 + 8.774X_3 \\ +0.061X_1^2 - 0.060X_1X_3 + 0.027X_2X_3 - 0.208X_3^2$$

For pre-treated seeds, the results were much simpler. For 180 ºC-treated seeds, only sonication time (X_2) and H/S ratio (X_3) showed significance. The reduced quadratic model that shows the relationship between the different factors and the oil recovery is:

$$Y = 47.045 + 0.226X_2 + 3.388X_3$$

In Figure 3, the main effects are presented graphically. It is clear that for native seeds, sonication temperature and H/S ratio led to variations in oil recovery. By examining the H/S ratio, it seems that recovery did not reach its maximum and that, at higher ratios, recovery would increase. This aspect needs further confirmation. This factor was also the most influential for treated seeds, reaching maximum recovery at close to 7. For 180 ºC-treated seeds, sonication time also produced significant increases in recovery, as addressed in Table 5.

In summary, the H/S ratio was the most influential factor, although in some cases it was the time and/or temperature during sonication treatments as well. This ratio is also discussed in most published works, and is identified as the most significant fac-

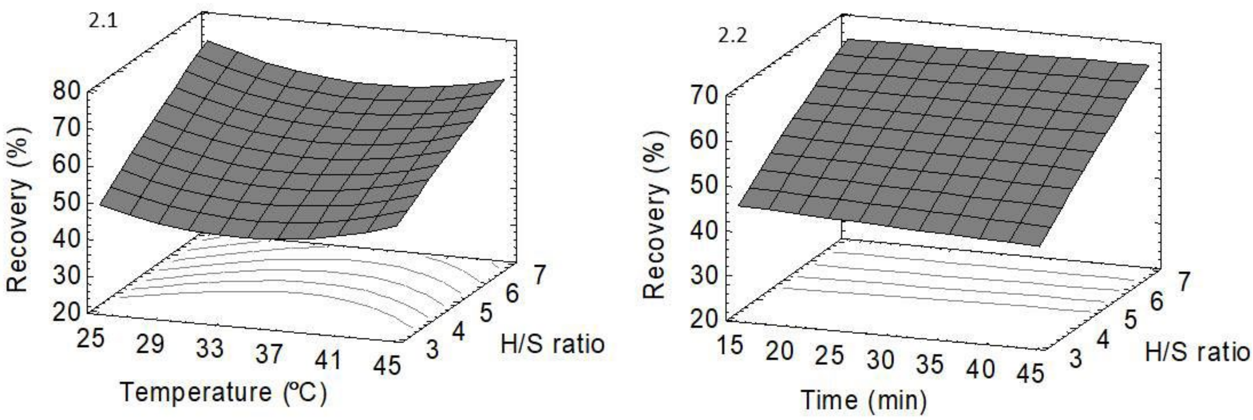

FIGURE 2. Response surface plots of interactions between sonication temperature (ºC) and hexane/solid ratio (2.1), and sonication time (min) and hexane/solid ratio (2.2) on oil extraction yield (%) for native date seed sample.

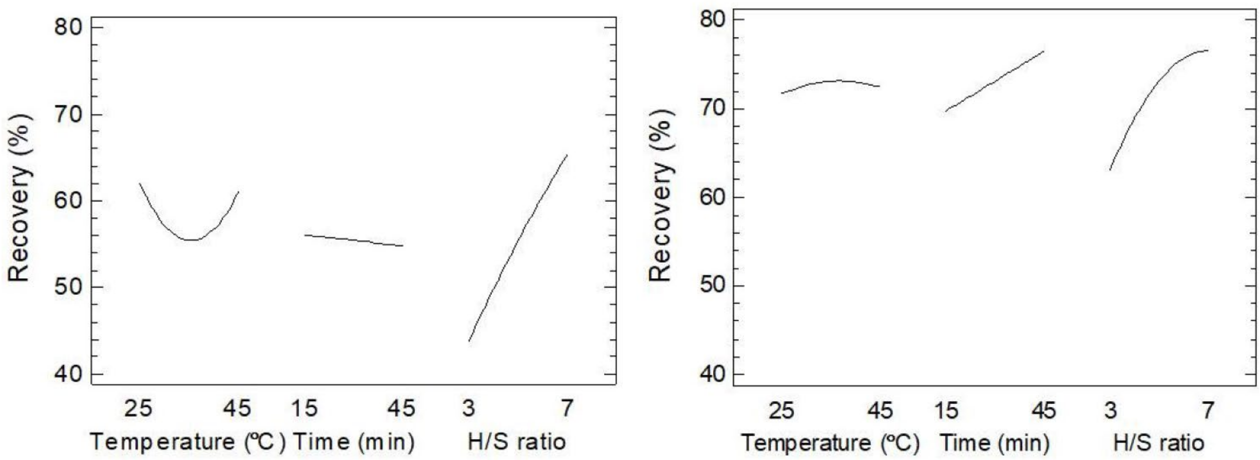

FIGURE 3. Main effect plots showing the effect of sonication temperature, sonication time and hexane/solid ratio on date seed oil recovery.

tor in the ultrasound-assisted extraction of oil from capers (Ara *et al*., 2014), black seeds (Abdullah and Koc, 2013), and rapeseed flakes (Perrier *et al*., 2017). Sonication time and temperature, and their interactions with the H/S ratio had different effects, depending on materials and other experimental conditions, as occurred in our study.

3.4. Optimization and validation of the different models presented

A statistical study of optimization was carried out to find the optimal extraction conditions for each sample. To validate the model, three experiments were developed at optimal conditions and a t-test was used to verify significant differences (confidence level 95%) between the predicted values and the experimental ones.

For native seeds, the highest predicted recovery (74.32%, i.e. 5.42% oil yield) was found at sonication temperature and time of 45 ºC and 15 min, and at the H/S ratio of 7. The experimental average was 71.03±2.33% (5.18±0.17% as oil yield). For treated seeds at 180 ºC, the highest recovery (80.93%, i.e. 6.71 oil yield) was found with 45 min sonication, at 35 ºC and H/S ratio of 7. A mean value of 79.28±0.83% recovery (6.58±0.07% oil yield) was obtained. In any case, there were no significant differences between predicted and experimental values for recovery percent, so the validity of the designed models was confirmed. The recovery increased with the hydrothermal pre-treatment, increasing from 71% in untreated date seeds to 80% in those treated at 180 ºC. This higher oil yield could be related to the disorganization of the seed matrix due to hydrothermal pre-treatment, which facilitated oil extraction.

Similar recoveries (70-80%) were found in the bibliography when working with black seeds, 74.77% (Abdullah and Koc, 2013) or grape seeds, 74.77-82.9% (Böger *et al*., 2018; Malicanin *et al*., 2014), the highest recoveries reported. Oils from winter melon (Bimakr *et al*., 2012) or pomegranate seeds (Goula, 2013) were recovered in lower amounts, 43.2 and 59.8%, respectively. For H/S ratio, most authors found that the bests conditions were at high ratios, e.g. 20 for kiwi and pomegranate seeds (Cravotto *et al*., 2011; Goula, 2013), or even higher (40 for rapeseed flakes (Perrier *et al*., 2017)). It is clear that the volume of hexane used for extraction has a positive influence on oil recovery, but some authors pointed to its negative effect on the oxidative stability of the oil (Samaram *et al*., 2015). Sonication temperature and time at optimum conditions were very similar to ours, varying between 20 and 50 ºC and 20 and 45 min.

3.5. Oxidative stability of extracted oils

As pointed out throughout this work, ultrasound could affect the oil's oxidative stability under certain conditions. To confirm the influence of ultrasounds on the stability of date seed oil, an assay was developed in which the absorbance at 540 nm was

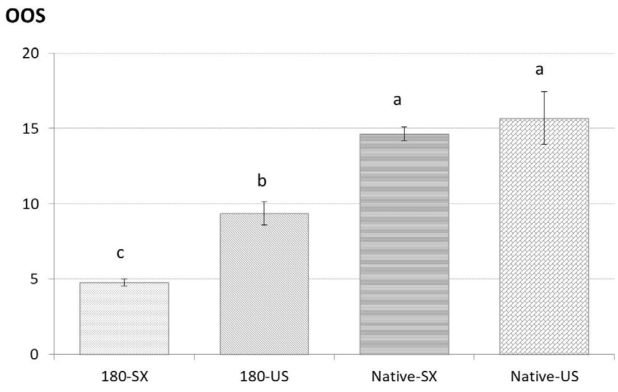

FIGURE 4. Oxidative stability of optimized oils from native date seeds and seeds treated at 180 ºC. The extractions were performed by Soxhlet (SX) or ultrasound-assisted (US). The results are the average values of four replicates. Different letters mean significant differences at $p < 0.05$.

inversely proportional to oxidative stability. The compounds resulting from oil oxidation (aldehydes, ketones, etc.) are TBA-reactive species, therefore unstable oils are easily oxidized at TBA assay conditions and give high 540 nm absorbance values. The results for OOS are shown in Figure 4. For native seeds, no differences were found between Soxhlet and UAE oils. The extractions at relatively low H/S ratio, as presented in this work, could maintain oil stability better than when extracted at higher ratios (>10) (Samaram *et al*., 2015).

Significant decreases in oil stability were quantified in the case of 180 ºC-treated seeds. These results suggested that, in any case, ultrasounds did not lead to a loss in stability when compared to Soxhlet, but hydrothermal pretreatment did. With 180 ºC pretreatment, the oil recovered by both sonication and Soxhlet was less stable. The higher resistance to oxidation in UAE oils from pre-treated seeds (180-US) when compared to its corresponding Soxhlet oil (180-SX) could be due to a more effective extraction of antioxidant molecules which are originated during the hydrothermal pre-treatment of date by-products (Mrabet *et al*., 2015). Pre-treatments could also destroy the naturally present antioxidants in date seed oil, but this loss could be partially counteracted by the appearance of new antioxidant compounds during hydrothermal treatments.

4. CONCLUSIONS

The extraction of oil from date seeds can be a profitable way to give added value to this by-product from date fruit processing industries. By applying environmentally friendly technologies (hydrothermal treatments and sonication), oil yield would be close to that obtained by traditional extraction methods (Soxhlet). With sonication alone, a recovery of nearly 75% was achieved, working at 45 ºC for 15 min, instead of 8 h at 70-80 ºC (Soxhlet conditions). In addition, the volume of solvent is much lower in assisted extractions (optimized H/S ratio near 7) than in the traditional ones (H/S ratio 20) and, under these conditions, the OOC did not decrease, probably due to the low H/S ratio. These results suppose savings in working time, energy and solvent, which would counter the lower sonication extraction yields. Higher recoveries were reached when working with pre-treated seeds, but the oil's stability decreased. From these results, the sonication-assisted extraction of untreated seeds seemed to be the most adequate system for developing an energy- and solvent-saving process, with valuable extraction yield and good oil stability. It would be interesting to study the fatty acid composition and bioactive compounds present in the oil (tocopherols, sterols, and phenols) in order to monitor the effects of the applied treatments to the chemical composition of the oils. Knowledge about how the different parameters affect oil composition and stability is of great interest for the industrial application of the proposed technology.

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