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Effect of screw-press extraction process parameters on the recovery and quality of pistachio oil

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SUMMARY: Pistachio nuts have high economical and nutritional value, mostly due to their elevated oil content (50%), which is composed mainly of oleic and linoleic fatty acids. Box-Behnken experimental designs were performed to optimize the oil extraction by response surface analysis. The independent variables measured were seed moisture content (SMC), restriction die (RD), screw press speed (PS) and pressing temperature (PT), while the response variables considered were oil recovery (OR), fine solid contents in the oil (FSC), oil remaining in the cake (OC) and parameters related to oil quality (free fatty acid composition (FFAC, mg KOH/g oil), peroxide value (PV, meq O_2/kg oil), K_{232}, K_{270} and pigment contents, mg/kg oil). Given that the chemical quality of pistachio nut oil pressed under different conditions was not affected, the process response was optimized in order to maximize OR under two pressing temperatures. Therefore, at 75 °C, pistachio oil extraction showed a maximum OR (79.61 g/100 g oil) at 8% SMC, 4 mm RD and 20 rpm SP; while, under cold-pressed conditions, the maximum OR (65.97 g/100 g oil) was achieved at 10% SMC, 4mm RD and 20 rpm SP. It is important to highlight that OR values were higher than the results reported previously and the chemical quality parameters from both oils were in the range of Codex standards for virgin (non-refined) oils (FFAC < 0.31 and PV < 0.33).

KEYWORDS: *Chemical quality parameters; Oil recovery; Pistachio oil; Screw-press extraction*

RESUMEN: ***Efecto de los parámetros del proceso de extracción por prensado sobre el rendimiento y la calidad de aceite de Pistacho.*** Los frutos de pistacho son altamente nutritivos, principalmente debido a su contenido de aceite (50%), compuesto principalmente por ácidos grasos mono y di-insaturados. Con la finalidad de conocer las condiciones de proceso adecuadas que permitan maximizar la cantidad de aceite extraído preservando la calidad química del mismo se utilizaron dos diseños experimentales de Box-Behnken. Las variables independientes analizadas fueron la humedad de la semilla (HS), el diámetro de reducción (DR), la velocidad y temperatura de prensado (VP y TP, respectivamente), mientras que las variables de respuesta fueron el rendimiento en aceite (RA), el contenido de sólidos finos en el aceite (CFA), el aceite remanente en torta (AT) y parámetros relacionados con la calidad (composición en ácidos grasos libres (CAGL, mg KOH/g aceite), índice de peróxido (IP, meqO_2/kg aceite), K_{232}, K_{270} y pigmentos (mg/kg aceite)). La calidad química de los aceites obtenidos no mostró daño oxidativo y/o hidrolítico significativo bajo las diferentes condiciones del proceso (IP< 0.33 y CAGL< 0.31, respectivamente), por lo tanto, la respuesta se optimizó para maximizar RA a dos TP, 75 y 40 °C. El rendimiento en aceite se maximizó ajustando HS (8% ó 10% b.h.), DR (4 mm), VP (20 rpm) a ambas temperaturas, alcanzando extracciones de aceite de 79.61% y 65.97%, respectivamente.

PALABRAS CLAVE: *Aceite de pistacho; Extracción por prensado; Parámetros de calidad química; Rendimiento de aceite*

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1. INTRODUCTION

Regarding the nutritional value of natural products, tree nuts rank third after fruits and spices for containing the most phytochemicals (Pérez-Jiménez *et al.*, 2010). Within these trees, the pistachio (*Pistacia vera* L.) is a member of the Anacardiaceae family, native to Central and Western Asia. Their fruits contain seeds that are widely consumed as snacks (roasted, natural or salted-roasted) and as components of several edible products (Martínez *et al.*, 2016). Thus, in recent years, this crop has been introduced in some countries in America, such as Argentina, Chile and in the United States. The total pistachio production is estimated at an average of 3500–4000 MT annually (Chang *et al.,* 2016).

Pistachio nuts are highly nutritious, mainly due their elevated lipid and protein contents (46 - 67 g/kg and 20 - 25 g/kg of nut weight, respectively) (Bullo *et al.,* 2015). These nuts also contain 14% carbohydrates, 1% fibers, minerals (mainly potassium), vitamins (β carotene, thiamine, riboflavin, tocopherols and ascorbic acid) and other water-soluble compounds, such as polyphenols and pigments, which have been associated with health benefits (Tsantili *et al.*, 2010; Schulze-Kaysers *et al.,* 2015).

In addition, many works highlight the quality of pistachio oil for use in food (Ventakatachalam and Sathe, 2006; Chahed *et al.*, 2008; Tsantili *et al.*, 2010; Ojeda-Amador *et al.*, 2018a) due its fatty acid profile which is composed mainly of oleic acid (52% to 72%) and linoleic acid (12% to 31%) (Arena *et al.*, 2007; Sena-Moreno *et al.*, 2015). The presence of minor bioactive compounds in pistachio oil, such as phytosterols and phenols, has also been reported previously (Tomaino *et al.*, 2010).

Previous works showed that pistachio oil recovery and composition, such as polyphenol and tocopherol contents, were affected by the extraction process (Sena Moreno *et al.*, 2015). These authors concluded that solvent extraction was the best o among the methods analyzed. However, it is important to note that the oil obtained by this process presented lower sensory and chemical qualities due to the presence of undesirable flavors and the reduction in the contents of vitamins and phenolic compounds (Abdolshahi *et al.*, 2015). Supercritical fluids is an alternative method for replacing or complementing conventional industrial oil extraction processes such as solvent extraction, and permits the extractions to be carried out at lower temperatures, achieving high oil recovery with good chemical quality (Jokic *et al.*, 2014). However, the heightened costs associated with processing are the greatest limitation associated with this extraction method.

In this context, the screw-press extraction technique is as an alternative process to solvent removal, especially for specialty oils because it provides an easy and reliable method of processing seeds and nuts with high oil contents (Wiesenborn *et al.*, 2001; Singh *et al.*, 2002; Zengh *et al.*, 2003; Martínez *et al.*, 2008; 2012; 2013; 2017a; 2107b). There are previous studies in which pistachio oil extraction by pressing was analyzed (Alvarez-Ortí *et al.*, 2012; Sena Moreno *et al.*, 2015; Ling *et al.*, 2016; Rabadán *et al.*, 2017; Ojeda-Amador *et al.*, 2018b). Particularly, Rabadán *et al.*, (2017) and Alvarez-Ortí *et al.*, (2012) compared hydraulic and screw-press, and concluded that a higher oil recovery (70 and 40%, respectively) was obtained by screw pressing regardless of the processing conditions. The oils did not present differences in chemical quality parameters in wither of the evaluated methods. Ojeda-Amador *et al.,* (2018b) mainly studied the influence of several processing conditions, including roasting, temperature, restriction die and screw-press speed, on the minor components contained in virgin pistachio oils. Sena-Moreno *et al.*, (2015) evaluated the effect of the drying temperature of pistachio nuts on the sensory and physicochemical characteristics of the oils, as well as the chemical composition. Finally, Ling *et al.*, (2016) analyzed the effects of different roasting treatments applied before screw pressing on oil quality.

Nevertheless, it is important to highlight that these authors did not make a systematic study of all the variables that influence the screw-press extraction efficiency (oil recovery and fine solids co-extracted with the oil) or the chemical quality of the oils obtained under each processing condition.

Screw-press performance strongly depends on the type of raw material (Mattea 1999, Martínez and Maestri, 2015) and the conditioning methods. The extraction process parameters for a given raw material must be determined due the fact that it is not feasible to assume a similar behavior even when comparing among nuts or oilseeds. Regarding the method for preparing the raw material, it consists of a number of unit operations such as cooking, cracking, flaking, moistening or drying, among others (Wiesenborn *et al.*, 2001; Martínez *et al.*, 2008; 2012; 2013; Savoire *et al.*, 2013; Martínez and Maestri, 2015; Martínez *et al.*, 2017a; 2017b). In fact, seed moisture content appears to be a key process variable that should be determined for each given nut or oilseed (Singh and Bargale, 1990; 2000; Hamm and Hamilton, 2000; Singh *et al.*, 2002; Martínez *et al.*, 2008; 2012; 2013; 2017a; 2017b). Singh and Bargale (1990, 2000) have observed maximum oil recoveries at 7% (wet basis) moisture content for water-soaked linseed, and at 7.5% for rapeseed, in a moisture content range of 5–12% (wet basis). Martínez *et al.*, 2008, 2012, 2013 and 2017b, reported that moisture content was one of the most important factors affecting walnut, chia, almond and sesame oil extraction, with 7.5, 10, 8, and 12.3% (wet basis) moisture as the optimal values, respectively.

Considering that several factors may significantly influence the extraction efficiency and the oil chemical quality in oil pressing and extraction processes, the response surface methodology is often applied in order to evaluate the individual and combined effect of each process parameter on the pressing process and quality of the oil (Akinoso and Raji 2011; Martínez *et al.*, 2012; Martínez and Maestri 2015).

Taking into account the above cited, the objective of the present work was to analyze the effect of extraction parameters of raw pistachio nuts on oil recovery (OR) and physical-chemical quality through screw-pressing. The experimental designs included different levels of the process variables, seed moisture content (SMC), pressing temperature (PT) and speed (PS), and restriction die (RD) in order to optimize OR and oil quality in a pilot scale screw press.

2. MATERIALS AND METHODS

2.1. Nut materials and moisture content

Pistachio nuts (*Pistacia vera* L. cv. Kerman) were harvested at full maturity from commercial plantations in the San Juan Province, Argentina. The nuts were cleaned, ground and screened to reach a particle size between 2.4 to 4.8 mm. The initial moisture content was determined according to official methods (AOCS, 2009) and, in order to obtain the moisture content levels indicated for each experimental design, the nuts were hydrated following the methodology proposed by Martinez *et al.*, (2008). The water-sprinkled samples were stored for 2 days before use in an air-tight metal container (500 g) to reach moisture equilibration. During this time the containers were shaken at regular intervals to distribute moisture uniformly throughout the sample. To regulate lower moisture content levels, the nuts were kept in a vacuum oven at 25 °C until the defined moisture was reached. The nuts contained 48.51 ± 1.85% oil (Dry Basis: DB) and 3.79 ± 0.23% initial moisture content (Wet Basis: WB).

2.2. Screw-press extraction

Oil extraction was carried out in a single step with a Komet screw press (Model CA 59G, IBG Monforts, Mönchengladbach, Germany). The press barrel had an effective and total length and an internal diameter of 3.1, 7, and 3.5 cm, respectively. The screw had a length and diameter of 15 and 3 cm, respectively. The first Box-Behnken experimental design included three levels of each following parameters: restriction die (RD: 4, 5 and 6 mm); seed moisture content (SMC: 3, 7 and 11% WB); pressing temperature (PT: 25, 50 and 75 °C); and pressing speed (PS: 20, 40 and 60 rpm). After each run, all press devices were conditioned. The amount of nut sample pressed in each run was 200 g. The screw-press components and the sample were first heated for 30 min to achieve the desired temperature. Temperature was constantly monitored during extraction with a digital thermometer (TES Thermometer 1307 Type K) inserted into the restriction die.

2.3. Oil recovery and fine solids content

The oil recovery (OR) was calculated taking into consideration the initial oil content in the raw material (48.51%) and the oil remaining in cake (OC). It was expressed as g extracted oil/g oil initial x 100 (g/100 g oil). In both cases, oil contents were determined by Soxhlet extraction using *n*-hexane as solvent (AOCS, 2009).

The extracts obtained from each run were centrifuged at 13.000 rpm for 15 min. The precipitated solids were recovered, washed with *n*-hexane, dried and weighed. Fine solid content (FSC) was expressed as g solids/100 g extract (oil + solid) (Martínez *et al.*, 2012).

2.4. Oil analysis

2.4.1. Chemical quality parameters and fatty acid composition

Free fatty acid content (FFAC) expressed as mg KOH/g oil, peroxide value (PV) expressed as meq O_2/kg oil, and specific extinction coefficients (K_{232} and K_{270}) were quantified according to standard methods (AOCS, 2009).

Fatty acid (FA) composition was analyzed according to procedures reported earlier (Maestri *et al.*, 2015) using gas chromatography (GC) (Perkin-Elmer, Shelton, CT, USA). The identification of FA was made by comparing their retention times with those of reference compounds and expressed as relative percentage (%).

2.4.2. Minor compounds and oxidative stability

The total phenol content (TPC) was determined by the Folin–Ciocalteau method according to Torres *et al.*, (2009) using 20 g of oil and quantified by comparison of the absorbance value (725 nm) with those from a standard curve with gallic acid (GA). TPC was expressed as mg GA/kg oil. The quantification and identification of tocopherols were carried out using high pressure liquid chromatography (Perkin-Elmer, Shelton, CT, USA) according to AOCS methods (Ce 8–89, 2009) with some modifications. Samples of 1 g pistachio oil were placed in 10-mL volumetric flasks. The required amount of *n*-hexane was added and swirled to dissolve into the sample. An aliquot of 20 μL of this solution was injected onto a Supelcosil LC-NH2-NP column (25 cm x 4.6 mm, Supelco, Bellefonte, PA, USA). The mobile phase was *n*-hexane/ethyl acetate (70/30 v/v) with a flow rate of 1 mL/min. UV detection at 295 nm was performed. Individual tocopherols were identified by comparison of their retention times with those of authentic standards (CN Biomedicals, Costa Mesa, CA) and were quantified by the external standard method. Finally, pigments were measured spectrophotometrically according to Minguez-Mosquera *et al.,* (1991). Both minor components were expressed as mg/kg oil.

The oxidative stability index (OSI) was measured by the Rancimat (Metrohm, Herisau, Switzerland) method (Cd 12b-92 AOCS, 2009). The assay conditions were: 3.5g oil; air flow rate of 20 L/h; and a temperature of the heating block of 110 ºC. The results were expressed as induction time (h), which corresponds to the break point in the plotted curves.

2.5. Experimental design and response surface analysis

Response surface methodology (RSM) was selected to model and optimize the extraction conditions for pistachio nut oil (Montgomery, 2005; Akinoso and Raji, 2011; Martínez *et al*., 2012; 2017a; 2017b; Martínez and Maestri, 2015). A Box-Behnken design was used to identify interactions among process parameters and response variables, as well as those conditions that optimized the oil extraction process.

First, an exploratory experimental array was carried out in order to define the range of process variables (Table 1). Three different levels were used for each of the following factors: seed moisture content (SMC) (X_1: 3,7 and 11%); restriction die (RD) (X_2: 4, 5 and 6 mm); pressing temperature (PT) (X_3: 25, 50 and 75 °C); and pressing speed (PS) (X_4: 20, 40 and 60 rpm). In this preliminary assay the responses evaluated were only oil recovery (OR) (g/100 g oil) (Y_1) and fine solid contents (FSC) (g solids/100 g extract) (Y_2).

Based on the conclusions determined from the preliminary test and so as to optimize the experimental conditions of the oil extraction process, a new Box-Behnken experimental design was made. In this second test the factors that significantly affected the OR were included. The evaluated responses in this design were: OR (g/100 g oil) (Y_1); FSC (g solids/100 g extract) (Y_2), oil remaining in cake (OC) (g/100 g oil) (Y_3); peroxide value (PV) (meq O_2/kg oil) (Y_4); free fatty acid content (FFAC) (mg KOH/g oil) (Y_5); specific extinction coefficients K_{232} (Y_6) and K_{270} (Y_7); lutein content (LC) (mg/kg oil) (Y_8); and chlorophyll content (CC) (mg/kg oil) (Y_9).

In both Box-Behnken experimental designs, quadratic polynomials were fitted in order to express the responses (Y_n) as a function of factors (X_i); where the response is Y, the constant term is β_0, the coefficients of the linear parameters are represented by β_i, the factors are represented by X_i, the coefficients of the quadratic parameter are represented by β_{ii}, and the coefficients of the interaction parameters are represented by β_{ij}.

$$Y = \beta_0 + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{3} \beta_{ii} X_i^2 + \sum \sum_{i<j}^{3} \beta_{ij} X_i X_j \quad \text{Eqn. (1)}$$

Two different designs were performed, the first one as exploratory (preliminary essays) and the second one, to define the optimal combination among the process extraction conditions. In both designs, the results were analyzed by a multiple regression method. The regression models were obtained from the experimental results. Performance of the model fitness was evaluated by ANOVA (Statgraphic Plus software v 5.1, USA). The coefficient of determination, R^2, explains the extent of the variance in a modeled variable that can be explained by the model. Only models with high R^2 were incorporated into this study. Only significant coefficients ($p < 0.05$) were included in the multiple regression equations. Three-dimensional response-surface graphics were created for each response variable. The determinations were made at least in duplicate, randomly, and replicas of the central point were performed to permit estimation of pure error as square sums. Statistical analysis of data was done by Statgraphic Plus software (v5.1, USA).

3. RESULTS AND DISCUSSION

In the first part, an experimental design of 28 treatments was developed (4 central points) with factors and levels previously described (Table 1). It is important to note that for the run with conditions 2, 6, 7 and 10, no extract was obtained. These results could be due to the combination of a low seed moisture content (SMC) and high pressing speed (PS), which did not provide the adequate conditions for pressing so the oil was not able to flow and leave the matrix. With regards to oil recovery (OR), the maximum value (66.53 g/100 g oil) was obtained in extraction 11 (Table 1), with a seed moisture content (SMC) of 7%, a restriction die (RD) of 4 mm, a pressing speed (PS) and temperature (PT) of 20 rpm and 50 °C, respectively. Treatments with SMC of 7% showed the highest variability (11.36-66.53 g/100 g oil). The ANOVA analysis determined that SMC ($p = 0$), PT ($p = 0$) and RD ($p = 0.0414$) showed p-values lower than the level of significance ($p < 0.05$); whereas PS did not have significant influence ($p = 0.0554$) (Table 2).

Figure 1 shows the individual effects of the process variables on OR. There were certain levels of SMC and PT which led to maximum OR values.

TABLE 1. Box-Behnken exploratory assay. Effects of extraction variables on oil recovery (OR: g/100 g oil) and fine solid contents (FSC:g solids/100 g extract) in oil. X_1: seed moisture (%, WB), X_2: restriction die (mm), X_3: pressing temperature (°C) and X_4: pressing speed (rpm).

	Factors				Response variables	
Assay	X_1	X_2	X_3	X_4	**OR**	**FSC**
1	7	5	25	20	18.67 ± 1.03	6.86 ± 0.49
2	7	5	25	60	ND	ND
3	7	4	25	40	18.59 ± 1.11	6.57 ± 0.45
4	7	6	25	40	11.36 ± 0.87	6.18 ± 0.39
5[b]	7	5	50	40	45.01 ± 2.54	7.39 ± 0.4
6	3	5	25	40	ND	ND
7	3	6	50	40	ND	ND
8	3	4	50	40	7.09 ± 0.76	8.46 ± 0.51
9	3	5	50	20	14.17 ± 1.65	10.32 ± 0.55
10	3	5	50	60	ND	ND
11	7	4	50	20	66.53 ± 3.04	4.38 ± 0.36
12	7	6	50	20	55.86 ± 1.54	5.1 ± 0.41
13[b]	7	5	50	40	49.30 ± 2.04	5.81 ± 0.43
14[b]	7	5	50	40	53.88 ± 2.01	5.76 ± 0.47
15[b]	7	5	50	40	50.51 ± 2.32	6.63 ± 0.45
16	7	4	50	60	56.07 ± 1.89	6.58 ± 0.42
17	7	6	50	60	40.64 ± 2.97	6.61 ± 0.37
18	7	4	75	40	61.83 ± 2.99	6.89 ± 0.47
19	7	6	75	40	40.66 ± 2.76	7.19 ± 0.48
20	3	5	75	40	22.94 ± 1.89	10.57 ± 0.43
21	11	5	25	40	39.68 ± 1.34	4.34 ± 0.38
22	11	4	50	40	58.86 ± 1.78	5.59 ± 0.42
23	11	6	50	40	45.54 ± 2.43	4.35 ± 0.37
24	11	5	50	20	58.03 ± 2.76	3.79 ± 0.29
25	11	5	50	60	55.01 ± 1.98	7.39 ± 0.48
26	7	5	75	60	48.99 ± 2.55	7.28 ± 0.37
27	11	5	75	40	55.03 ± 2.43	4.86 ± 0.34
28	7	5	75	20	57.09 ± 2.76	6.34 ± 0.38

[b] Central points. Mean values (± standard deviation) were the averages of two independent measurements. ND: not detected.

However, in the case of RD and PS, negative effects were observed, since less compression of material was generated and a shorter residence time of the material inside the pressing chamber resulted in reductions in OR. For walnut oil, similar trends for SMC were obtained at pilot and industrial scale extraction conditions (Martínez *et al*., 2008; 2017a). By increasing SMC from 2.5 to 7.5% and 5.5 to 10% (WB) these authors observed an increase in walnut OR at pilot scale extractions from 61 to 83.5% (at 25 °C) and from 64.7 to 89.3% (at 50 °C); and at industrial scale extraction from 44.8 to 80.4% (at 35 °C). The positive effect of increasing water content on OR may be explained through an increasing expansion and breaking of cell structures as well as its lubricating action which expedites oil release (Li *et al*., 1999).

With respect to fine solid content (FSC) the ANOVA analysis revealed that PT significantly affected (p = 0.0275) FSC, while the other factors (SMC, RD and PS) had no significant effect (p > 0.05). Nevertheless, it is important to note that the highest values for FSC were obtained from samples 9 (10.32 g solid/100 g extract) and 20 (10.57 g solid/100 g extract) with a SMC of 3% and a PT of 50 °C and 75 °C, respectively (Table 1). While at higher SMC (7% and 11%) the extracts presented lower FSC (4.34-7.39 g solid/100 g extract). According to Vargas-López *et al.,* (1999), Singh *et al.,* (2002) and Martínez *et al.,* (2008) the FSC decreased when the SMC increased due the press cake becoming more compact, causing a smaller amount of sediment to pass through the barrel orifices to be co-extracted

TABLE 2. Values of regression coefficients calculated for pistachio oil recovery in both designs. (X1: seed moisture content, X2: restriction die, X3: pressing temperature and X4: pressing speed)

Independent variable	Regression coefficient	Significance level (p)
First Design		
Constant	-166.812	
X_1	20.198 ± 2.52	0.0000
X_2	22.354 ± 2.52	0.0414
X_3	3.30736 ± 2.52	0.0000
X_4	-0.37116 ± 2.52	0.0554
X_1^2	-0.93669 ± 4.80	0.0020
X_2^2	-1.65208 ± 4.80	0.6788
X_3^2	-0.02028 ± 4.80	0.0063
X_4^2	-0.00162 ± 4.80	0.8706
X_1*X_2	-0.3894 ± 6.55	0.7496
X_1*X_3	-0.01897 ± 6.55	0.6977
X_1*X_4	0.034844 ± 6.55	0.5696
X_2*X_3	-0.1394 ± 6.55	0.4787
X_2*X_4	-0.0595 ± 6.55	0.8072
X_3*X_4	0.00528 ± 6.55	0.5896
R^2		91.23
Second Design		
Constant	94.3745	
X_1	11.4494 ± 2.26	0.2395
X_2	-39.8581 ± 2.26	0.0021
X_3	0.906219 ± 2.26	0.3337
X_1^2	-0.52094 ± 3.19	0.2396
X_2^2	2.95375 ± 3.19	0.1137
X_3^2	-0.00028 ± 3.19	0.9455
X_1*X_2	0.49625 ± 3.19	0.5570
X_1*X_3	-0.07725 ± 3.19	0.1010
X_2*X_3	-0.00862 ± 3.19	0.9175
R^2		86.60

with oil. In fact, the SMC had a quadric effect on FSC (Figure 2).

Figure 2 also shows that when RD and PS increased, a reduction in FSC was observed due to a decrease in the compression degree of the material and a shorter residence time inside the pressing chamber. However, in all treatments evaluated, FSC showed low values, which was considered a positive trait since it facilitated subsequent operations of oil clarification. Therefore, considering that PS did not affect significantly OR and that the maximum OR coincided with the extreme SMC treatments (7 and 11%), a new experimental design was made in order to find the extraction conditions which maximized OR (Table 3).

The new experimental design was elaborated considering the following factors and levels: SMC (X_1: 8, 10 and 12%); PT (X_2: 25, 50 and 75 °C); and RD (X_3: 4, 5 and 6 mm) in order to determine an adequate combination to maximize OR and minimize FSC. The PS was kept constant at 20 rpm because in these types of fruits, with high oil content, low pressure speeds are recommended in order to increase the time of the material permanence in the pressing chamber (Martinez *et al.*, 2017a). This is in concordance with Rabadán *et al.* (2017), who found that low PS (17 rpm) increased oil yields, while high PS (96 rpm) led to low oil yields. A new Box-Behnken design with 16 experiments and four central points was developed to study the effect of these incoming factors. The experimental design and the response variables considered (OR, FSC, OC, PV, FFAC, K_{232}, K_{270}, LC and CC) are shown in Table 3.

The statistical analysis showed that RD significantly affected OR ($p < 0.05$); while SMC and PT had no significant effects ($p > 0.05$) (Table 2). Equation of the fitted model is:

$$OR = 94.37 + 11.44\, X_1 - 39.86\, X_2 + 0.906\, X_3 - 0.521\, X_1^2 + 2.954\, X_2^2 - 0.0003\, X_3^2 + 0.496\, X_1 {*}\, X_2 - 0.077\, X_1 {*}\, X_3 - 0.0086\, X_2 {*}\, X_3$$

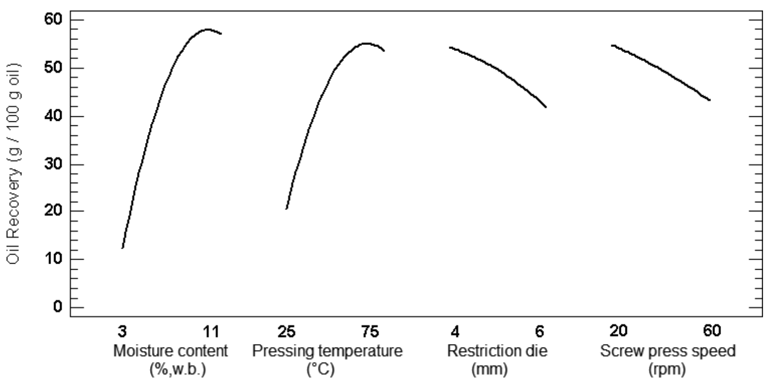

FIGURE 1. Main significant effects on oil recovery in pistachio oil extraction for preliminary Box- Behnken assay (First Design)

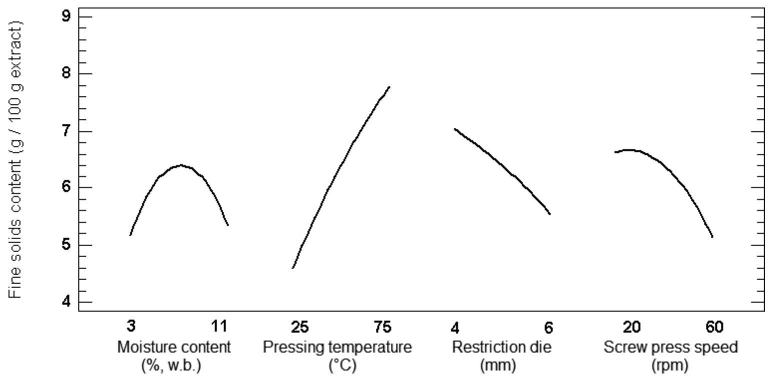

FIGURE 2. Main significant effects on fine solid contents in oil in pistachio oil extraction for preliminary Box- Behnken assay (First Design).

The coefficient of determination of the model (R^2) was able to explain 86.6% of the data variability.

Figure 3 shows that a decrease in RD caused an increase in OR, reaching a maximum value of approximately 70.88 g/100 g of oil. On the other hand, although the effect of PT on OR was not significant, a directly proportional relationship was observed between the variables. SMC had a negative and positive quadratic effect on OR. At low SMC values OR was enhanced; while at high SMC the opposite effect was observed. This result is in agreement with Martinez *et al.,* (2017a), who observed that walnut and almond oil extraction at high SMC (12%) negatively affected OR due to insufficient friction during pressing.

It is important to highlight that the second experimental design (Table 3) enhanced OR values (53.61 - 70.88 g/100g oil) compared to the preliminary assay (7.09 a 66.53 g/100 g oil) (Table 1). In comparison with other works, Ling *et al.,* (2016) obtained OR in the range of 32.95-33.27 g/100 g oil from roasted kernels and a value of 31.57 g/100 g oil from raw kernels. On the other hand, Ortiz-Moreno *et al.,* (2015) reported a maximum OR value with a screw press close to 40 g/100 g oil. Finally, Rabadán *et al.,* (2017) reported OR to be approximately of 70 g/100g oil using the screw press at 75 °C and 17 rpm and Ojeda-Amador *et al.,* (2018b) reported the best performance at 130 °C (68.5 and 68.8 g/100 g) for Larnaka and Kerman pistachio varieties. Regarding the chemical quality of pistachio oil, Table 3 shows that the main chemical parameters were within the limits accepted by CODEX (1999) for virgin cold-pressed oils (PV < 15 meq O_2/ kg oil and FFAC < 4 mg KOH/g oil). PV and FFAC values varied from 0.05 - 0.28 meq O_2/ kg oil and 0.21 - 0.33 mg KOH/g oil, showing the absence of oxidative and hydrolytic deterioration. Specific extinction coefficient (K_{232} and K_{270}) values were within the ranges of 1.6 and 1.8 and 0.14 and0.16, respectively. These data show that the different extraction treatments had minimal effects on chemical quality parameters and oxidative stability.

Finally, lutein content (LC) was between 37.55-48.51 mg/kg oil, while chlorophyll content (CC) was between 18.3 and 26.37 mg/kg oil. These values were lower than those for pistachio oil reported by Martínez *et al*., (2016), who observed amounts between 41.4-69.6 and 48.5-57.5 mg/ kg oil for LC and CC, respectively. According to Bellomo and Fallico (2007), pigment contents were strongly influenced by the degree of maturity and the origin of pistachio nuts.

In summary, different process conditions for pistachio oil extractions had negligible effects on the chemical quality parameters determined; however, OR was highly affected. The combination of factor levels which suggests maximum OR (70.88 g/100 g oil) and a low FSC (7.72g solids/100 g extract) is in acconcordance with treatment number 3 (8% SMC, 4 mm RD and 50 °C PT) (Figure 4).

According to the desirability function the combination of process variables that optimized a maximum OR (75.87 g/100g oil) were: 8% SMC; 75 °C PT; and 4 mm RD. This arrangement of factors was conducted according to the procedures mentioned above. Table 4 shows that no significant differences were found between the values predicted by the model (75.87 g/100 g oil) and the experimental value observed (79.61 ± 2.56 g/100g oil) for OR which proved a good fit of the model to the experimental data.

However, in order to obtain a cold-pressed pistachio oil, a new experimental design was made by adjusting PT to 40 °C according to CODEX definitions (CODEX, 1999). In this case, the combination of each factor level which predicts a maximum OR (69.89 g/100 g oil) within the experimental values was 10% SMC, 40 °C PT and 4 mm RD. Table 4 shows that no significant differences were found between the value estimated by the model (69.89 g/100 g oil) and the experimental value (65.97 ± 3.06 g/100g oil) for OR, which indicates a good fit of the model to the experimental data. This value is higher than the OR reported by Ojeda-Amador *et al.,*

TABLE 3. Box-Behnken assay (Second Design). Effect of process variables (X_1: seed moisture (%, WB), X_2: pressing temperature (°C), X_3: restriction die (mm)) on oil recovery (OR: g/100 g oil), fine solid contents in oil (FSC: g solids/100 g extract), oil remaining in cake (OC: g/100 g oil), oil quality parameters (Peroxide value, PV: meq O_2/kg oil; Free fatty acid content, FFAC: mg KOH/g oil, K_{232} and K_{270}), luteolin content (LC: mg/kg oil) and chlorophyll content (CC: mg/kg oil)

	Factors			Yield and oil quality parameters								
Assay	X_1	X_2	X_3	OR	FSC	OC	PV	FFAC	K_{232}	K_{270}	LC	CC
1	8	75	5	65.58 ± 1.89	8.14 ± 0.67	22.08 ± 1.02	0.27 ± 0.01	0.25 ± 0.01	1.79 ± 0.003	0.16 ± 0.003	48.51 ± 2.34	26.92 ± 1.54
2	8	50	6	53.61 ± 1.05	8.31 ± 0.77	29.85 ± 1.45	0.24 ± 0.01	0.22 ± 0.01	1.66 ± 0.002	0.15 ± 0.002	41.74 ± 2.13	20.41 ± 1.76
3	8	50	4	70.88 ± 1.78	7.72 ± 0.65	19.99 ± 1.56	0.28 ± 0.02	0.26 ± 0.01	1.72 ± 0.002	0.16 ± 0.002	44.86 ± 4.21	23.19 ± 2.02
4[b]	10	50	5	60.90 ± 2.03	5.46 ± 0.46	24.30 ± 1.78	0.18 ± 0.01	0.23 ± 0.01	1.65 ± 0.001	0.15 ± 0.003	44.50 ± 2.44	23.44 ± 1.78
5[b]	10	50	5	62.66 ± 1.76	7.14 ± 0.67	22.71 ± 1.93	0.18 ± 0.02	0.25 ± 0.02	1.66 ± 0.006	0.15 ± 0.004	44.64 ± 3.23	24.20 ± 2.45
6[b]	10	50	5	62.51 ±1 2.59	6.09 ± 0.56	21.69 ± 1.65	0.27 ± 0.01	0.22 ± 0.01	1.66 ± 0.01	0.15 ± 0.008	43.91 ± 2.32	22.20 ± 1.08
7	12	25	5	57.23 ± 1.78	6.58 ± 0.76	25.79 ± 1.99	0.2 ± 0.01	0.27 ± 0.02	1.79 ± 0.002	0.15 ± 0.002	40.92 ± 1.77	21.47 ± 1.74
8[b]	10	50	5	61.88 ± 2.54	8.23 ± 0.78	20.40 ± 1.54	0.18 ± 0.01	0.27 ± 0.02	1.64 ± 0.002	0.15 ± 0.005	42.17 ± 1.89	21.41 ± 1.88
9	10	75	4	69.92 ± 2.31	7.09 ± 0.65	20.26 ± 1.76	0.25 ± 0.01	0.27 ± 0.02	1.70 ± 0.009	0.16 ± 0.002	47.76 ± 3.55	24.62 ± 2.24
10	12	50	4	70.11 ± 1.67	6.34 ± 0.48	21.98 ± 1.56	0.27 ± 0.01	0.27 ± 0.01	1.74 ± 0.002	0.15 ± 0.007	45.99 ± 2.76	25.12 ± 2.45
11	12	75	5	54.88 ± 1.98	7.64 ± 0.67	27.01 ± 1.89	0.26 ± 0.01	0.33 ± 0.03	1.67 ± 0.007	0.16 ± 0.002	45.31 ± 1.67	25.41 ± 1.43
12	10	75	6	60.78 ± 2.08	7.93 ± 0.59	24.09 ± 2.01	0.27 ± 0.02	0.27 ± 0.01	1.68 ± 0.014	0.16 ± 0.010	47.19 ± 2.54	26.37 ± 2.32
13	12	50	6	56.94 ± 1.76	7.96 ± 0.61	28.00 ± 2.15	0.28 ± 0.02	0.26 ± 0.01	1.71 ± 0.011	0.16 ± 0.005	46.38 ± 1.87	26.24 ± 2.44
14	10	25	4	68.69 ± 1.88	7.12 ± 0.48	24.42 ± 1.76	0.26 ± 0.01	0.25 ± 0.01	1.63 ± 0.007	0.15 ± 0.001	40.38 ± 1.56	20.56 ± 1.01
15	8	25	5	58.21 ± 1.67	8.27 ± 0.66	29.53 ± 2.02	0.05 ± 0.01	0.21 ± 0.01	1.64 ± 0.009	0.15 ± 0.002	37.55 ± 2.67	18.29 ± 1.32
16	10	25	6	60.26 ± 2.05	6.73 ± 0.56	29.24 ± 1.87	0,28 ± 0.02	0.22 ± 0.01	1.59 ± 0.013	0.14 ± 0.002	38.02 ± 2.78	20.08 ± 1.27

Mean values (± standard deviation) were the averages of two independent measurements.
b Central points

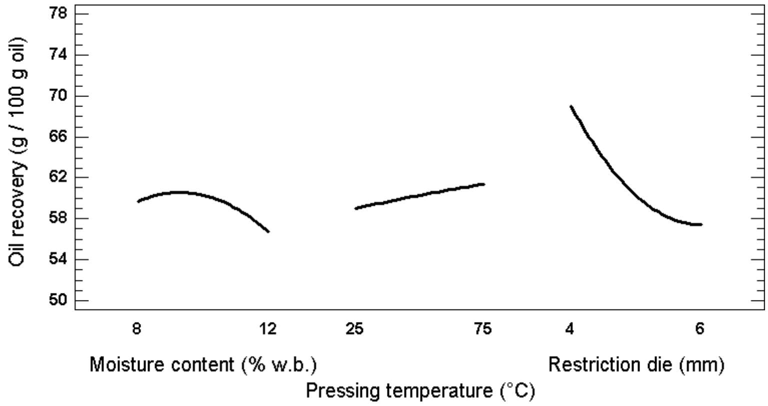

FIGURE 3. Main significant effects on oil recovery in pistachio oil extraction (Second Design).

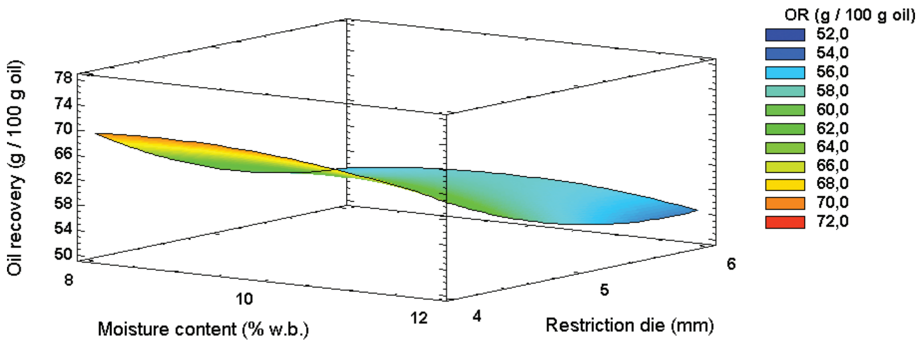

FIGURE 4. Effects of seed moisture content and restriction die on pistachio oil recovery at 50 °C pressing temperature (Second Design).

(2018b) for Larnaka and Kerman pistachio varieties (56.1 and 40.1 g/100 g oil, respectively) under a similar extraction temperature (40 °C). Moreover, the results demonstrated that even if the PT did not exert a significant influence on OR, it was observed in both optimizations that if this variable is modified, the SMC change in order to maximize OR. Additionally, SMC and PT modified the seed permeability and oil viscosity, respectively.

Table 4 presents the chemical parameters of the oil acquired from the optimized responses. As explained previously, the oil chemical quality (PV, FFAC, k_{232} and k_{270}) was not negatively affected by the screw press extraction process. The oils obtained under both extraction conditions presented OSI values similar as those reported by Rabadán *et al.*, (2017) (30.91 h). Regarding FAAC, the order of abundance was as follows: oleic acid (18: 1) > linoleic acid (18: 2) > palmitic acid (16: 0) > αLinolenic acid (18: 3) > palmitoleic acid (16: 1) > stearic acid (C18: 0). Oleic acid was the major fatty acid, representing more than 50% of the total FA present in pistachio oil. These results are in agreement with those reported by Arena *et al.*, (2007), Chahed *et al.*, (2008) and Ojeda-Amador *et al.,* (2018a) from Kerman and other pistachio varieties.

With respect to pistachio oil tocopherol composition, the first authors to report it were Kornsteiner *et al.*, (2006) and Miraliakbari and Shahidi (2008). Although these authors partially agreed on the total tocopherol contents (298 and 334 mg/kg oil, respectively) in solvent extracted oils (hexane), the proportion of the individual components markedly differs between them. While Kornsteiner *et al.*, (2006) reported major amounts of β and γ isoforms; Miraliakbari and Shahidi (2008) identified α-tocopherol as the most abundant. In the present work the obtained oil had a total tocopherol content of 1000 mg/kg oil (Table 4), similar to the value obtained by Ojeda-Amador *et al.,* (2018b) from roasted Larnaka pistachio oil (830 mg/Kg), but much higher than Ling *et al.*, (2016) who reported a maximum of 367 mg/Kg oil. In acconcordance with these works γ-tocopherol was the most abundant isomer found and in agreement with Ojeda-Amador *et al.*, (2018b), the increase in temperature during the extraction process did not significantly favor tocopherol solubility in oil.

Table 4: Chemical parameters of oils extracted under processing conditions for maximum oil recovery (20 rpm and 4 mm of restriction die) with: A) 10% seed moisture content and 40 °C pressing temperature, B) 8% seed moisture content and 75 °C pressing temperature (OR: oil recovery, FSC: fine solid contents, OC: oil remaining in the cake, PV: peroxide value, FFAC: free fatty acid content, OSI: oxidative stability index)

Optimal extraction conditions (20 rpm; 4mm)		
	A (10%;40 °C)	**B (8%;75 °C)**
Oil yield parameters		
OR (g/100 g oil)	$65.97^a \pm 3.06$	$79.61^b \pm 2.56$
FSC (g solids/100 g extract)	$10.32^b \pm 1.23$	$7.39^a \pm 0.89$
OC (g/100 g oil)	$26.69^b \pm 2.54$	$18.36^a \pm 1.05$
Oil quality parameters		
PV (meq O_2/kg oil)	$0.26^a \pm 0.03$	$0.33^a \pm 0.03$
FFAC (mg KOH/g oil)	$0.24^a \pm 0.01$	$0.31^a \pm 0.02$
K_{232}	$1.72^a \pm 0.03$	$1.67^a \pm 0.09$
K_{270}	$0.156^a \pm 0.001$	$0.156^a \pm 0,001$
OSI (h)	$30.34^a \pm 1.78$	$31.81^a \pm 1.56$
Main fatty acids (%)		
Palmitic (16:0)	$11.55^a \pm 0.89$	$11.60^a \pm 1.78$
Palmitoleic (16:1)	$0.97^a \pm 0.23$	$0.883^a \pm 0.08$
Stearic (18.0)	$0.37^a \pm 0.02$	$0.614^a \pm 0.02$
Oleic (18:1)	$51.90^a \pm 1.09$	$54.65^a \pm 2.01$
Linoleic (18:2)	$33.10^a \pm 1.56$	$31.90^a \pm 1.65$
Linolenic (18:3)	$2.17^a \pm 0.34$	$0.95^a \pm 0.10$
Tocopherols content (mg/kg oil)		
α-Tocopherol	$115^a \pm 4.14$	$113^a \pm 2.10$
β-Tocopherol	$54^a \pm 2.06$	$55^a \pm 1.11$
γ-Tocopherol	$777^a \pm 35.3$	$786^a \pm 16.0$
δ-Tocopherol	$61^a \pm 1.32$	$62^a \pm 1.97$
Pigments content (mg/kg oil)		
Luteolín	$43.53^a \pm 0.92$	$47.24^a \pm 3.22$
Chlorophyll	$22.33^a \pm 1.05$	$26.93^a \pm 2.34$
Total Phenol content (mg GA/kg oil)	$10.30^a \pm 0.69$	$11.28^a \pm 0.78$

Mean values (± standard deviation) were the averages of two independent measurements. Values in each row with different superscript letters present significant differences ($p < 0.05$) among treatments (Fisher Test).

With respect to the total phenol contents (TPC) (Table 4), both extracted oils presented a lower TPC (10.3-11.28 mg GA/Kg oil) than those reported by Rabadán *et al.,* (2017) (24.31 mg GA/kg oil), Ling *et al.,* (2016) (38.7-49.5 mg GA/kg oil) and by Ojeda-Amador *et al.,* (2018b) (approximately 16-76 mg GA/kg).These differences among studies can be attributed to the fact that the TPC of nuts are affected not only by the genotype, but also the orchard location, harvest year, maturity index, processing, and storage conditions (Bolling *et al*., 2011). In addition, it has been reported (Ojeda-Amador *et al.,* 2018b) that the solubility of phenolic compounds increases in the oil phase but at higher temperatures (between 80 °C and 140 °C) than those used in this study. The same authors have reported that this high temperature affects the oil pigment content, but a rise is not evident in this work between both temperatures studied (Table 4).

4. CONCLUSIONS

The Box-Behnken experimental design was employed to optimize pistachio oil extraction through screw-pressing operations. Seed moisture content, pressing temperature and restriction die resulted in the main processing parameters. The different combinations of process parameters did not generate oxidative and/or hydrolytic damage in the obtained oils; however, oil recovery was significantly affected. Oil recovery from pistachio seeds by pressing can be maximized by modifying moisture content (8% or 10%, w.b.), pressing temperature (75 °C or 40 °C), 4 mm restriction die and 20 rpm screw-press speed to obtain oil recoveries of 79.61% or 65.97% of the total oil available, respectively.

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