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Analytical Methods

Response surface optimization of ultrasound-assisted oil extraction from autoclaved almond powder

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ABSTRACT

An investigation into ultrasound-assisted extraction and autoclaving pretreatment was conducted for the oil extraction from almonds. The best possible combination of extraction parameters was obtained with the response surface methodology (RSM), at a three-variable, three-level experiment Box–Behnken design (BBD). The optimum extraction parameters were as follows: extraction time, 55 min; extraction temperature, 51 °C; and solvent/sample ratio, 19:1, at a fixed ultrasonic frequency of 40 kHz and power of 150 W. Under these conditions, the oil recovery was 81.89 ± 0.23% for the autoclaved almonds, which well matches with the predicted value. Furthermore, the oil composition was analyzed with GC–MS, and the effect of the autoclaving on the oil extraction was evaluated. The results showed that the autoclaving pretreatment increased the oil recovery, without affecting the oil composition, by 8.69%, which confirmed the efficacy of the autoclaving on the oil extraction from almond powder.

1. Introduction

Almond (*Prunus amygdalus*) is one of the most popular tree nuts on a worldwide basis. Its seeds are typically used as snack foods and as ingredients in a variety of processed foods, notably bakery and confectionery products (Dourado, Barros, Morta, Coimbra, & Gama, 2004).

Almonds contain ~20% (w/w) protein and, except for methionine, provide all of the essential amino acids in quantities equal to or greater than those recommended by the FAO guidelines (Esteban, López-Andréu, & Carpena, 1985; Saura-Calixto, Bauza, Martinez de Toda, & Argamenteria, 1981). Apart from the high protein content, the almonds also contain as much as about 50% oil. As one of the most popular vegetable oils, it is rich in mono- and polyunsaturated fatty acids, with oleic and linoleic acids as the major constituents, and contains the naturally occurring Vitamins A, B_1, B_2, B_6 and Vitamin E. This characteristic composition of the almond oil makes it a valuable material for the food industry.

The almond oil is also widely used in aromatherapy for giving body massage. The oil, highly absorbable, serves as a great emollient, helping make so gentle, comfortable a touch to the skin. It helps keep the balance of the moisture in the body, suitable for all skin types. It also makes a great lubricant, thus aiding in combating itchings and inflammatings. Owing to the multiple benefits of the almond oil, the interest in it has greatly increased in recent years (Femenia, García-Marín, Simal, Rosselló, & Blasco, 2001; López-Ortiz et al., 2008; Marrone, Poletto, Reverchon, & Stassi, 1998; Martín-Carratalá, Llorens-Jordá, Berenguer-Navarro, & Grané-Teruel, 1999; Sharma & Gupta, 2006; Wang, Zhang, Guo, & Zhang, 2004).

As the oilseed is a cell assembly with a hard shell of the cell wall, the oil exists in the oil body of the cell (Cheng & Song, 2006). Therefore, the breaking of all the cells is needed before the oil is obtained, and cellulase, hemicellulase and protease can generally assist in the extraction recovery (Kasai, Imashiro, & Morita, 2003). However, after the cell wall is broken, the protein and the oil seep out simultaneously, forming an emulsion of oil and water that are difficult to isolate from each other (Kasai et al., 2003). To solve this problem, Kasai et al. (2003) developed an efficient method with the pretreatment using autoclaving and enzymes to prepare single cell for the soybean oil extraction. However, there is no report, to the best of our knowledge, on the almond oil extraction with the help of the autoclaving treatment.

In the case of the almond oil extraction, a great variety of new approaches based on different principles have been developed. Sharma and Gupta (2006) reported the ultrasonic pre-irradiation effect upon aqueous enzymatic oil extraction from almond. The ultrasonic pre-irradiation enhanced the yields from 77% to 95% (w/w). However, the method demanded three specific proteases

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and cost at least 6 h. Marrone et al. (1998) studied the almond oil extraction with supercritical carbon dioxide fluid (SCF-CO_2). Based on the principle of "broken" and "intact" cells, they proposed an extraction model which predicted an asymptotic value about 90% (w/w) for the oil recovery with an extraction time more than 10 h. Femenia et al. (2001) evaluated the main effect of SCF-CO_2 on the cell wall of almond seed in oil extraction, while much attention was focused on the fundamental aspects of SCF extraction processes. Therefore, a time-saving, economical and simple process for the almond oil extraction would be of industrial interest.

In this paper, the ultrasonic extraction parameters such as extraction time, extraction temperature, and solvent/sample ratio, were optimized with the response surface methodology (RSM) employing a three-variable, three-level Box–Behnken design (BBD), for the oil extraction from the autoclaved almond powder. Furthermore, the effect of the autoclaving pretreatment on the oil extraction was evaluated in the aspects of almond powder microstructure and oil recovery.

2. Materials and methods

2.1. Materials

The almonds were obtained from the local market in Yulin City, Shaanxi Province, China. All the chemicals and solvents used were of analytical grade.

2.2. Sample preparation and autoclaving treatment

The almond seeds were ground into powder with a cyclone mill and passed through a 60 mesh sieve. The autoclaving treatment was carried out using the method described by Kasai et al. (2003) with some modifications. The almond powder was dipped in five times of water at 4 °C overnight. After being filtered through Whatman No. 1 paper (Whatman-Xinhua Filter Papers Co., Zhejiang, China), two times of water was again added to the powder for the purpose of promoting the adhesive substances between the almond seed cells (e.g. glycine or hydroxyproline-rich protein or galacturonic polysaccharides) to be transferred to the water, and the powder was autoclaved at 121 °C for 10 min, then immediately depressured to zero to destroy the hard and compact honeycombed pericarp of the almond seed. The autoclaved almond powder was filtered and then dried with air, and stored at the low temperature for future use.

2.3. Soxhlet extraction

Soxhlet extraction lasted 8 h in duplicate for 15 g of the almond powder (6.2 g water/100 g almond seeds) with 250 ml of hexane, and the hexane was removed with a rotary vacuum evaporator. The oil was then dried in an oven at 85 °C to constant mass (Luque-García & Luque de Castro, 2004). Soxhlet extraction gave a yield of 54.80 g oil/100 g almond seeds, which was taken as 100% in measuring the rate of the oil recovery by the ultrasound-assisted extraction from the autoclaved almond powder.

2.4. Ultrasound-assisted extraction

The ultrasound-assisted extraction was performed in an ultrasonic cleaning bath (KQ3200B type, 40.0 kHz, 150 W, Kunshan ultrasonic instrument Co., Ltd., Jiangsu, China) with a usable capacity of 2.5 l (the internal dimensions: 30.0 × 15.0 × 15.0 cm). An in-water pipe was added to the opposite of out-water pipe in the bath, and the flux ratio between in-water and out-water was regulated to keep solution temperature stable in the test.

Autoclaved samples were placed into a conical flask (150 ml), made up to required volume with hexane, and sonicated at required temperature for different times. Then the mixture was filtered through Whatman No. 1 paper under the condition of vacuum, and the solvent was removed with a rotary vacuum evaporator at 50 °C. The oil yield was calculated according to the weight, and the recovery was expressed as percentages of the Soxhlet yield.

2.5. Experimental design and statistical analysis

A three-variable, three-level Box–Behnken design (BBD) (Box & Wilson, 1951; Wanasundara & Shahidi, 1996; Wang, Sun, Cao, Tian, & Li, 2008; Yu, Dandekar, Toledo, Singh, & Patil, 2007) was applied to optimizing the extraction condition in order to obtain the high oil recovery from the autoclaved almond powder. The three independent variables set were extraction time (min, X_1), extraction temperature (°C, X_2), and solvent/sample ratio (ml/g, X_3), and each variable set at the three levels. A total of 17 experiments were designed (Table 1). Each experiment was performed in triplicate and the average oil recovery (%) was taken as the response, Y.

Regression analysis was performed for the experiment data and was fitted into the empirical second order polynomial model, as shown in the following equation:

$$Y = \beta_0 + \sum_{i=1}^{3} \beta iXi + \sum_{i=1}^{3} \beta ii X_i^2 + \sum_{i=1}^{2} \sum_{j=i+1}^{3} \beta_{ij} X_i X_j \quad (1)$$

Where, β_0, β_i, β_{ii} and β_{ij} are regression coefficients in the intercept, linear, quadratic, and interaction terms, respectively; X_i and X_j are the independent variables.

A software Design-Expert 7.1.3 Trial (State-Ease, Inc., Minneapolis MN, USA) was used to obtain the coefficients of the quadratic polynomial model. The quality of the fitted model was expressed by the coefficient of determination R^2, and its statistical significance was checked by an *F*-test.

2.6. Gas chromatography–mass spectrometry analysis

Gas chromatography–mass spectrometry (GC–MS) analysis was performed with a SHIMADZU QP2010 instrument and SHIMADZU ChemStation software (SHIMADZU corporation analytical and measuring instruments division, Kyoto, Japan). A fused silica

Table 1
Box–Behnken design and observed responses[a].

Run	Independent variable			Response (Y%)
	X_1 (time, min)	X_2 (temperature, °C)	X_3 (solvent/sample, ml/g)	
1	40 (−1)	40 (−1)	15 (0)	69.40
2	60 (+1)	40 (−1)	15 (0)	74.86
3	40 (−1)	60 (+1)	15 (0)	74.45
4	60 (+1)	60 (+1)	15 (0)	78.34
5	40 (−1)	50 (0)	10 (−1)	77.05
6	60 (+1)	50 (0)	10 (−1)	78.33
7	40 (−1)	50 (0)	20 (+1)	79.12
8	60 (+1)	50 (0)	20 (+1)	79.98
9	50 (0)	40 (−1)	10 (−1)	71.18
10	50 (0)	60 (+1)	10 (−1)	72.45
11	50 (0)	40 (−1)	20 (+1)	75.83
12	50 (0)	60 (+1)	20 (+1)	78.83
13	50 (0)	50 (0)	15 (0)	81.96
14	50 (0)	50 (0)	15 (0)	79.95
15	50 (0)	50 (0)	15 (0)	81.92
16	50 (0)	50 (0)	15 (0)	80.98
17	50 (0)	50 (0)	15 (0)	81.35

[a] Average value of triplicate experiments.

capillary SHIMADZU RTX-5 ms (5% phenyl methyl siloxane) column (30 × 0.25 mm i.d., film thickness 0.25 μm) was used for the separation. Injector and ion source temperature were set at 260 and 200 °C, respectively. Oven temperature was raised from 184 to 191 °C by a rate of 0.2 °C/min. A 2 μl aliquot of oil was injected into the column at a split ratio of 10:1.

Helium was used as a carrier gas at a flow rate of 1.30 ml/min. The mass spectrometer was operated in electron-impact ionization (EI) mode with 70 eV energy. The scanning range was 40–500 amu and the scanning rate was 0.2 s per scanning.

The individual identification of components was based on the matching of their recorded mass spectra with those of NIST05.LIB and NIST05s.LIB (National Institute of Standards and Technology) libraries data provided by the software of GC–MS system.

2.7. Electron microscopy scanning

Samples were mounted on bronze stubs with double-sided adhesive tape allowing surface visualization, and then coated with a layer of gold (40–50 nm) in a sputter coater to avoid charging under the electron beam. A scanning electron microscopy spectrometer (Quanta-200, Philips-FEI Company, Amsterdam, Netherlands) was used at the operating voltage of 20 kV and the vacuum of 15 Pa. The high resolution topographic images were digitally recorded with the short dwelling times to prevent the beam induced damages. Measurements were taken in triplicate for each sample.

3. Results and discussion

3.1. Determining levels for independent variables

The three levels of the extraction time variable were determined according to the results of a series of experiments carried out for 20, 30, 40, 50, 60, 70, and 80 min at the extraction temperature of 40 °C and the solvent/sample ratio of 15:1 (ml/g). When the extracting time varied from 20 to 50 min, a remarkable increase of the oil recovery was observed. Beyond that time range, there was little difference observed in the recovery. Therefore, 40, 50, and 60 min were chosen for the coded extraction time variable levels at −1, 0, and +1, respectively.

The effect of the extraction temperature under the sonication on the oil recovery was investigated at 30, 35, 40, 45, 50, 55, and 60 °C for the extraction time of 30 min and the solvent/sample ratio at 15:1. A significant increase of the oil recovery was observed over the extraction temperature range (30–60 °C), the oil recovery reaching the maximum of around 81% at 60 °C. Higher temperatures are beneficial to the solubility of the almond oil in the extracting solvent, and could accelerate the extracting process. However, increasing temperature will bring about not only the increase in costs in the view of industrialization but also lipids oxidation. The three design levels selected for the temperature variable were 40, 50, and 60 °C, respectively.

The effect of different ratios of the solvent/sample on the oil recovery was examined at 5:1, 10:1, 15:1, 20:1, 25:1, and 30:1 at 40 °C for 30 min in the process of the ultrasound-assisted extraction. The oil recovery significantly increased from 56.1% to 72.3% with the ratio of solvent/sample increasing from 5:1 to 20:1. This is consistent with the mass transfer principle. The driving force during the mass transfer is the concentration gradient between the solid and the bulk of the liquid, which is greater when a higher solvent/sample ratio is used (Herodež, Hadolin, Škergeta, & Knez, 2003; Pinelo, Rubilar, Jerez, Sineiro, & Nunez, 2005). However, when the ratio continued to increase, the oil recovery changed very little. Therefore, 10:1, 15:1, and 20:1 (ml/g) were selected as the three variable levels for the solvent/sample ratio.

Table 2
Estimated regression coefficients for the quadratic polynomial model and the analysis of variance (ANOVA) for the experimental results.

Parameter[a]	Estimated coefficients	Standard error	DF[b]	Sum of squares	*F* value	Prob > F
Intercept				*Model*		
β_0	81.23	0.72	1	216.31	9.26	0.0039
β_1	1.44	0.57	1	16.50	6.36	0.0397
β_2	1.60	0.57	1	20.48	7.89	0.0262
β_3	1.84	0.57	1	27.20	10.48	0.0143
β_{11}	−1.46	0.79	1	8.99	3.46	0.1051
β_{22}	−5.51	0.79	1	127.76	49.22	0.0002
β_{33}	−1.15	0.79	1	5.58	2.15	0.1861
β_{12}	−0.39	0.81	1	0.62	0.24	0.6410
β_{13}	−0.11	0.81	1	0.044	0.017	0.9000
β_{23}	0.43	0.81	1	0.75	0.29	0.6080
Lack of fit				3	15.45	7.56
Pure error				4	2.72	
R^2	0.9225		Adjusted R^2	0.8229		
C.V.%	2.08		PRESS	251.39		

[a] Coefficients refer to the general model.
[b] Degree of freedom.

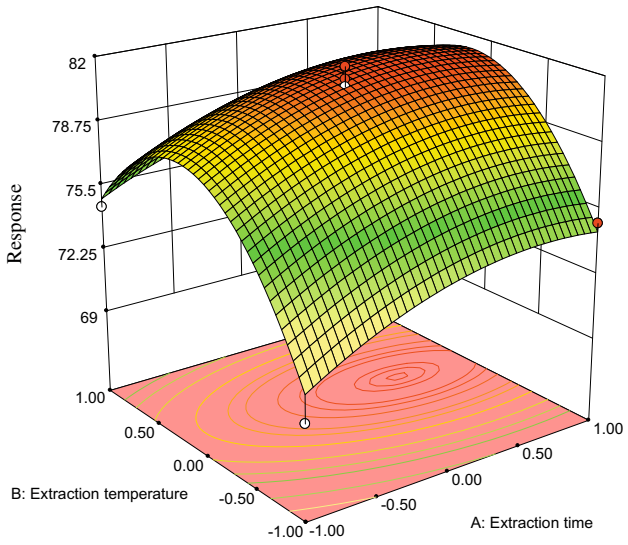
3.2. Response surface optimization of ultrasonic extraction condition

The condition for the ultrasound-assisted extraction of oil from the autoclaved almond powder was optimized using different variable combinations according to the Box–Behnken design (3^3 factorial). Table 1 presents the experiment design and corresponding response data for the oil recovery. The regression coefficients of the intercept, linear, quadratic, and interaction terms of the model were calculated using the least square technique and are presented in Table 2. It was evident that all the linear parameters and one quadratic parameter (extraction temperature) were found to be significant ($p < 0.05$ or $p < 0.01$), whereas all the interaction parameters were insignificant ($p > 0.1$). The results indicated that the effect of the extraction temperature was the major contributing factor to the oil recovery.

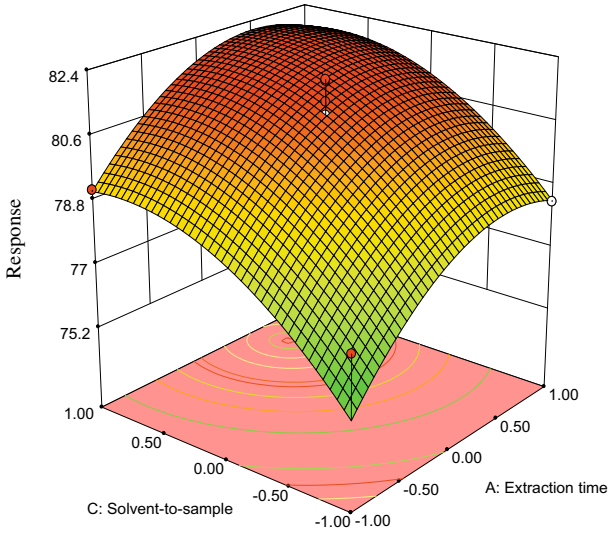
The analysis of variance for the experimental results of the Box–Behnken design is also shown in Table 2. The coefficient of determination (R^2) of the model was 0.9225, indicating that the model adequately represented the real relationship between the parameters chosen. Furthermore, results of the error analysis indicated that the lack of fit was insignificant ($p > 0.05$). The coefficient of variation (C.V.) of less than 5% indicated that the model was reproducible (Mason, Gunst, & Hess, 1989; Wanasundara and Shahidi, 1996). The Predicted Residual Sum of Squares (PRESS) for the model, which is a measure of how a particular model fits each point in the design, was 251.39. The model *F*-value, 9.26, implied that the model was significant. The predicted second-order polynomial model was:

$$Y = 81.23 + 1.44X_1 + 1.60X_2 + 1.84X_3 - 0.39X_1X_2 - 0.11X_1X_3 + 0.43X_2X_3 - 1.46X_1^2 - 5.51X_2^2 - 1.15X_3^2 \quad (2)$$

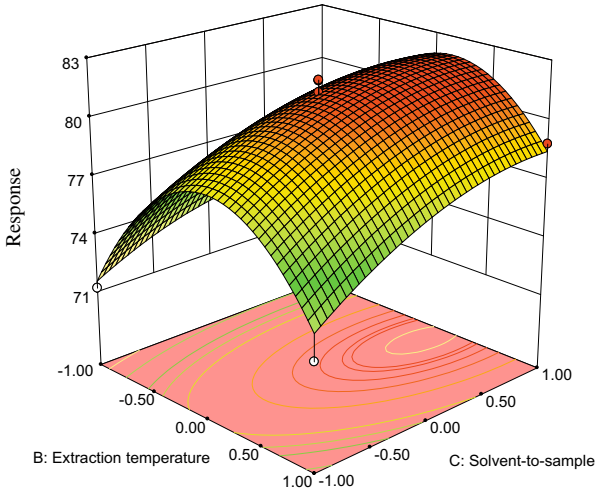
To determine optimal levels of the variables for the oil recovery from the autoclaved almond powder, the three-dimensional surface plots were constructed according to Eq. (2). Fig. 1a shows the effect of the extraction time and temperature on the oil recovery at a fixed solvent/sample ratio of 15:1. At a definite extraction temperature, the oil recovery increased slightly with the increase of the extraction time, and nearly reached a peak at the highest extraction time tested. However, the extraction temperature showed a quadratic effect on the response (oil recovery), and the maximum oil recovery was obtained at 51.4 °C, followed by a decline with the further increase of the extraction temperature. The interaction between the



(a) Solvent/sample ratio, 15:1 (ml/g)



(b) Extraction temperature, 50 ºC



(c) Extraction time, 50 min

Fig. 1. Response surface plots of the oil recovery affected by extraction temperature, extraction time, and solvent/sample ratio.

extraction time and solvent/sample ratio is shown in Fig. 1b at the fixed extraction temperature of 50 °C. The oil recovery increased with the extraction time and the solvent/sample ratio increasing, and the maximum oil recovery was obtained at 54.5 min and ratio of 18.9:1. Fig. 1c shows the effect of the extraction temperature and the solvent/sample ratio on the oil recovery at a fixed extraction time of 50 min. The results indicated that solvent/sample ratio displayed a linear effect on the response. The quadratic effect of the extraction temperature was striking, and the oil recovery reached the highest value also at 51.4 °C.

The optimal condition obtained using response surface methodology (RSM) was as follows: extraction time, 54.5 min; extraction temperature, 51.4 °C; and solvent/sample ratio, 18.9:1 (ml/g). To compare the predicted result (82.10%) with the practical value, the rechecking experiment was performed using this deduced optimal condition. The mean value of 81.89 ± 0.23% (n = 3), obtained from real experiments, demonstrated the validity of the RSM model, since there was no significant ($p > 0.05$) differences between 82.10% and 81.89 ± 0.23% (n = 3). The strong correlation between the real and the predicted results confirmed that the response model was adequate to reflect the expected optimization.

3.3. Effect of autoclaving pretreatment on oil extraction

In order to evaluate the effect of autoclaving pretreatment on the extraction of almond oil, the ultrasound-assisted extraction was also carried out for the almond powder without autoclaving. The low oil recovery of 73.20 ± 0.31% (n = 3) was obtained for the almond powder without autoclaving at the same extraction condition optimized above. Compared with the result of this low recovery, the autoclaving pretreatment made the oil extraction recovery increase by 8.69%, which confirmed the efficacy of the autoclaving pretreatment. Autoclaving (pressure and temperature) would solubilize and remove the adhesives between the cells of the almond seeds, which would greatly increase the available surface area between solvent and the cells, promote the oil recovery and reduce extraction time. The fast pressure swing during autoclaving treatment (i.e., the pressurization and depressurization steps) would partially disrupt the hard, compact and intricate honeycombed pericarp structure of the almond seed, especially, and then allow the penetration of solvent into the pericarp structure easily and cut down the extraction time from six or more hours mentioned above to less than 1 hour with a high oil recovery of 81.89%.

3.4. Fatty acid composition analysis of almond oil

The fatty acid composition analysis was carried out with GC–MS for the different almond oils obtained by the procedures of Soxhlet

Table 3
Fatty acid composition of the almond oils extracted with different procedures[a].

Fatty acid	Retention time (min)	SE (%)	UAE (%)	UAE-AP (%)
Myristic acid	4.53	1.09	1.47	1.31
Palmitoleic acid	7.76	0.81	0.76	0.36
Palmitic acid	8.32	8.05	7.42	6.55
Hexadecanoic acid	9.42	2.09	5.08	4.85
Phthalic acid	9.80	1.22	1.61	1.36
Linoleic acid	15.34	21.08	21.14	21.96
Oleic acid	15.69	62.80	59.15	60.28
Octadecanoic acid	15.95	1.91	2.06	2.06
Stearic acid	17.11	0.95	1.31	1.27

[a] Results are expressed as% over the total content (relative content); SE, Soxhlet extraction; UAE, ultrasound-assisted extraction; UAE-AP, ultrasound-assisted extraction in conjunction with the autoclaving pretreatment.

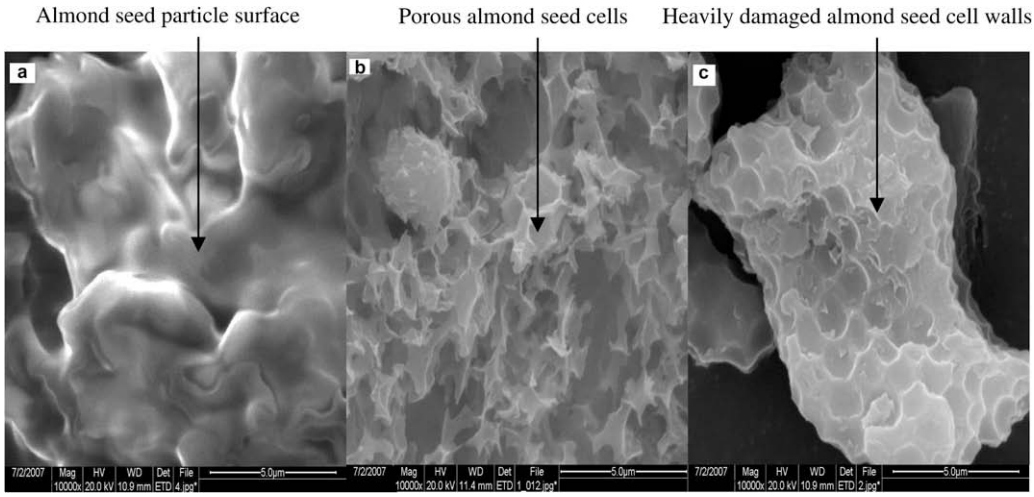


Fig. 2. Scanning electron micrographs of (a) non-irradiated, (b) ultrasonic irradiated, and (c) ultrasonic irradiated in conjunction with the autoclaving pretreatment of almond powder.

extraction (SE), ultrasound-assisted extraction (UAE), and ultrasound-assisted extraction in conjunction with the autoclaving pretreatment (UAE-AP), respectively. As shown in Table 3, all of the examined oils were very rich in the unsaturated fatty acids (oleic acid and linoleic acid making up from 80.29% to 83.88% of total fatty acids), and relatively low content in the saturated fatty acids. There were no appreciable differences among the oils obtained by the three extraction procedures, which shows that the oil composition is neither affected by the autoclaving pretreatment nor by the ultrasonic treatment.

3.5. Almond powder microstructure comparison

To gain further insight into the effect of the autoclaving pretreatment and the ultrasonic treatment on the almond oil extraction, the microstructure of the almond powder was analysed with scanning electron microscopy (SEM). Fig. 2a–c show the scanning electron micrographs of non-irradiated, ultrasonic irradiated, and ultrasonic irradiated in conjunction with the autoclaving pretreatment on the almond powder, respectively. Fig. 2b and c indicate that the almond powder became porous in morphology due to the structural breakage caused by the ultrasonic cavitating energy. In contrast, there were more cracks and more pores appearing in the almond powder with autoclaving pretreatment than that in the almond powder without autoclaving pretreatment. This consequence is reflected in the fact that the oil recovery increased by 8.69% from the almond powder with the autoclaving pretreatment.

4. Conclusions

In the present paper, the ultrasound-assisted extraction of oil from the autoclaved almond powder was performed with a three-variable, three-level Box–Behnken design (BBD) based on the RSM. The experiment results showed that the extraction temperature was the major contributing factor to the oil extraction. It was revealed that the ultrasonic and autoclaving pretreatment did not affect the composition of the almond oil, but the ultrasonic cavitating energy could cause structure breakage of the almond powder, and autoclaving pretreatment could accelerate this effect which increased the oil recovery by 8.69% and greatly reduced the extraction time.

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