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Optimisation of Microwave-Assisted Extraction of Pomegranate (*Punica granatum* L.) Seed Oil and Evaluation of Its Physicochemical and Bioactive Properties

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Summary

Pomegranate seed oil was extracted in a closed-vessel high-pressure microwave system. The characteristics of the obtained oil, such as fatty acid composition, free fatty acidity, total phenolic content, antioxidant activity and colour, were compared to those of the oil obtained by cold solvent extraction. Response surface methodology was applied to optimise extraction conditions: power (176–300 W), time (5–20 min), particle size (d=0.125–0.800 mm) and solvent to sample ratio (2:1, 6:1 and 10:1, by mass). The predicted highest extraction yield (35.19 %) was obtained using microwave power of 220 W, particle size in the range of d=0.125–0.450 mm and solvent-to-sample ratio of 10:1 (by mass) in 5 min extraction time. Microwave-assisted solvent extraction (MASE) resulted in higher extraction yield than that of Soxhlet (34.70 % in 8 h) or cold (17.50 % in 8 h) extraction. The dominant fatty acid of pomegranate seed oil was punicic acid (86 %) irrespective of the extraction method. Oil obtained by MASE had better physicochemical properties, total phenolic content and antioxidant activity than the oil obtained by cold solvent extraction.

Key words: pomegranate seed oil, microwave-assisted solvent extraction, extraction optimisation, bioactive properties

Introduction

Microwave-assisted solvent extraction (MASE) is a process that has emerged as an attractive alternative oil extraction method in recent years. The rapid heating and destruction of biological cell structure in a microwave provide more effective extraction in shorter time than conventional processes. Moreover, MASE requires small amount of solvent for extraction and produces high-quality oil regarding chemical and physical properties. Another important advantage of this process is the lower energy requirement resulting in a significant decrease in environmental impact and financial costs (*1–8*).

In the last decade, researchers have started to focus on the microwave-assisted extraction instead of the conventional methods for the extraction of natural compounds such as pectin, essential oil and phenolics. MASE has been extensively studied to investigate its impact on the extraction of high-value components with high yield and quality from various plant food materials, industrial food wastes and by-products. It has been widely used for the extraction of lycopene from tomato peel (*9*), polyphenols from red grape pomace, grape seed and potato peel (*10–12*), or essential oil and pectin from orange peel (*13*).

Pomegranate (*Punica granatum* L.) is an edible fruit cultivated in the Mediterranean area and Near and Far East countries (*1*). The edible part of whole fruit contains 75–85 % juice and 15–25 % seeds. Pomegranate seed is one of the most valuable food wastes mainly obtained from pomegranate juice industry (*14*). Pomegranate seed has 12–25 % crude oil which is rich in bioactive lipids

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(*15,16*). It contains tocopherols, phytosterols and punicic acid, which have several potential health benefits (*17*). Punicic acid constitutes 74–85 % of the total fatty acid content of pomegranate seed oil and is known for its antioxidant, antitumour, immunomodulatory, anti-atherosclerotic and serum lipid-lowering activities (*18,19*). Eikani *et al.* (*20*) compared the efficiency of Soxhlet, cold pressing and superheated hexane extraction methods in the extraction of pomegranate seed oil. Tian *et al*. (*21*) optimised the conditions for ultrasonic-assisted extraction of pomegranate seed oil. Fadavi *et al*. (*22*) investigated the total lipid content and fatty acid composition of pomegranate seed oil extracted from 25 different varieties grown in Iran. Sadeghi (*23*) evaluated the physical and chemical characteristics of four pomegranate cultivars. However, extraction of pomegranate seed oil has not been evaluated previously in a closed-vessel high-pressure microwave extraction system. The objectives of the presented study are to observe the effects of extraction time, solvent-to-solid ratio (by mass), particle size and microwave power on oil extraction yield in a microwave system using response surface methodology and to compare the yield and the quality parameters such as physical, chemical and bioactive properties of the oil obtained by MASE and cold solvent extraction.

Materials and Methods

Materials

The pomegranate (*Punica granatum* L. 'Hicaznar') seeds were provided by a fruit juice plant Göknur A. Ş., Niğde, Turkey. Folin-Ciocalteu phenol reagent and gallic acid were provided by Merck (Darmstadt, Germany). The 2,2-diphenyl-1-picrylhydrazyl radical (DPPH), sodium carbonate, hexane, methanol, and other solvents were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). All reagents and solvents were of analytical or chromatographic grade.

Sample preparation

The seeds were dried to moisture content of 3.5 % in a vacuum oven (model VD 23; Binder Inc., Bohemia, NY, USA) at 35 °C for 3 h. Dried seeds were ground in a coffee grinder (model PRG 259; Premier, Istanbul, Turkey). Ground particles were sieved through meshes into different particle sizes: fine particles d_1=0.125–0.450 mm (1), moderately coarse particles d_2=0.450–0.530 mm (2) and coarse particles d_3=0.530–0.800 mm (3), collected and used in the experiments. Ground particles were sealed in glass bottles and kept at 4 °C until extractions were performed.

Conventional extractions

Cold solvent and Soxhlet extraction methods were performed using the method proposed by Abbasi *et al*. (*2*) and Jiao *et al*. (*24*) with some modifications to compare their oil extraction efficiencies with that of microwave-assisted solvent extraction (MASE). Conventional Soxhlet extraction was performed using 10 g of fine powdered seeds (d_1=0.125–0.450 mm) and 220 mL of *n*-hexane in a classical Soxhlet apparatus at 110 °C for 8 h. For cold solvent extraction, 40 g of ground seeds and 400 mL of *n*-hexane were put into a glass beaker and stirred by magnetic stirrer (model 613.01.001; Isolab, Werthelm, Germany) with extraction time of 8 h at 25 °C. Finally, the solid residue was precipitated by centrifugation (3461×*g*, 10 min, EBA 20; Hettich, Tuttlingen, Germany). The supernatant was separated by decantation. Hexane was removed at 40 °C using rotary vacuum evaporator (Hei-VAP Advantage HL/G1; Heidolph Instrument GmbH & Co. KG, Schwabach, Germany) in both extraction methods. The extracted oil was stored at –20 °C until the analyses were carried out. The yield was determined using the following equation:

$$Y=\left(\frac{m(\text{extracted oil})}{m(\text{dry seeds})}\right)\cdot 100 \qquad /1/$$

Microwave-assisted solvent extraction

Microwave-assisted solvent extraction (MASE) was performed in a CEM Discover SP-D microwave reactor at 2450 MHz (CEM Corporation, Matthews, NC, USA). The extraction was checked and monitored *via* computer. Ground pomegranate seeds and *n*-hexane at different solvent-to-sample ratios (Table 1) were put into 35-mL microwave quartz vessel closed with snap-on caps. The values of power and time were adjusted as given in the experimental central composite design (Table 1) using CEM Synergy software (CEM Corporation). Stirring was set at high level. The extractions were performed in dynamic mode (maximum power *P*=300 W) and PowerMax function, which blows air to eliminate additional heating, was on. The required time for heating up and cooling down were not included in total extraction time. After extraction, the solid residue was precipitated by centrifugation (3461×*g*, 10 min) and supernatant was collected. Hexane was removed at 40 °C using rotary vacuum evaporator (Hei-VAP Advantage HL/G1; Heidolph Instrument GmbH & Co. KG). The extracted oil was stored at –20 °C prior to analysis. The yield was calculated using Eq. 1.

Experimental design and optimisation by response surface methodology

A three-level, four-factorial face-centred central composite design was applied to evaluate the effects of extraction parameters on oil extraction yield and to determine optimum extraction conditions for obtaining highest extraction yield. The design consisted of 30 experimental runs with six replications at the central point (Table 1). The extraction variables were time (5–20 min), power (176–300 W), solvent-to-sample ratio (2:1, 6:1 and 10:1) and particle size (d=0.125–0.800 mm). The response was the oil extraction yield (Y). The data were analysed as reported by Keskin *et al*. (*25*). The predicted values given by the model fitting technique in Design Expert v. 7.0 (Stat-Ease, Inc., Minneapolis, MN, USA) were closely correlated with the experimental values.

Determination of fatty acid composition

The fatty acid composition of the extracted oil was determined by using Agilent 7890A gas chromatograph (Agilent Technologies, Santa Clara, CA, USA) with a split/splitless injector, flame ionisation detector and HP-88 capillary column (88 % cianopropylaryl; 100 m×0.250 mm, 0.20 μm i.d.). The method proposed by Çiftçi *et al*. (*26*)

Table 1. A three-level, four factorial face-centred central composite design generated for microwave-assisted extraction of pomegranate seed oil

Run	Factors				*Y*/%	
	P/W	*t*/min	ζ(solvent, sample)	Particle size	Experimental	Predicted
1	176	5	2:1	1	25.32±0.01	25.96
2	300	5	2:1	1	26.03±0.03	25.96
3	176	20	2:1	1	27.33±0.11	27.41
4	300	20	2:1	1	27.56±0.12	27.41
5	176	5	10:1	1	35.14±0.07	35.19
6	300	5	10:1	1	35.29±0.08	35.19
7	176	20	10:1	1	36.21±0.16	36.64
8	300	20	10:1	1	36.51±0.07	36.64
9	176	5	2:1	3	7.88±0.09	8.07
10	300	5	2:1	3	9.67±0.12	8.07
11	176	20	2:1	3	7.65±0.13	7.97
12	300	20	2:1	3	7.60±0.05	7.97
13	176	5	10:1	3	10.19±0.06	10.48
14	300	5	10:1	3	10.06±0.08	10.48
15	176	20	10:1	3	10.66±0.16	10.38
16	300	20	10:1	3	11.52±0.08	10.38
17	176	12.5	6:1	2	25.16±0.19	25.43
18	300	12.5	6:1	2	25.38±0.06	25.43
19	238	5	6:1	2	24.80±0.08	25.09
20	238	20	6:1	2	25.42±0.06	25.77
21	238	12.5	2:1	2	20.44±0.05	20.62
22	238	12.5	10:1	2	26.27±0.08	26.44
23	238	12.5	6:1	1	34.26±0.17	33.20
24	238	12.5	6:1	3	9.71±0.25	11.13
25	238	12.5	6:1	2	24.95±0.31	25.43
26	238	12.5	6:1	2	26.32±0.12	25.43
27	238	12.5	6:1	2	25.67±0.18	25.43
28	238	12.5	6:1	2	25.01±0.25	25.43
29	238	12.5	6:1	2	26.20±0.15	25.43
30	238	12.5	6:1	2	25.82±0.16	25.43

All values are expressed as mean±standard deviation of three replicates.
Particle size: d_1=0.125–0.450 mm (1), d_2=0.450–0.530 mm (2) and d_3=0.530–0.800 mm (3)

was used with some modifications. The injector and detector temperatures were 250 and 260 °C, respectively. The oven temperature was scheduled as follows: 1 min at 120 °C, from 120 to 175 °C at 10 °C/min, 10 min at 175 °C, from 175 to 210 °C at 5 °C/min, 5 min at 210 °C, from 210 to 230 °C at 5 °C/min and 5 min at 230 °C. Helium was the carrier gas with a flow rate of 1.5 mL/min.

Determination of free fatty acid and total phenolic content, and peroxide value

The free fatty acid content and peroxide value of oil were determined according to AOCS Ca 5a-40 (*27*) and Cd 8-53 (*28*) methods, respectively. The total phenolic content of the extracted oil was determined using the Folin-Ciocalteu colourimetric method proposed by Gutfinger (*29*) with some modifications. The oil obtained by microwave-assisted or cold solvent extraction (2.5 g) was dissolved in *n*-hexane (5 mL). Oil-in-hexane solution together with 5 mL of methanol and water (60:40, by volume) mixture was vortexed to extract the phenolic compounds. Centrifugation at 3461×*g* for 10 min (EBA 20; Hettich) was used to separate the two phases. This extraction procedure was done in triplicate. The extracts in methanol were mixed and 0.2 mL of the methanolic phase was diluted to 5 mL with distilled water. Folin-Ciocalteu reagent (0.5 mL) was added to this mixture. After 3 min, 1 mL of Na_2CO_3 (20 %, by mass per volume) was added to the reaction mixture, which was diluted to a final volume of 10 mL with distilled water and held for 1 h in the dark. The absorbance values were measured against a blank sample at 765 nm using Lambda 25 UV/Vis spectrophotometer (PerkinElmer, Shelton, CT, USA). The calibration curve was obtained using gallic acid standard solutions (0–60 mg /mL). The results were expressed in mg of gallic acid equivalents per g of sample dry mass.

Antioxidant activity assay

The antioxidant activity of the oil was determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical with the method proposed by Kalantzakis *et al*. (*30*). Briefly, 4 mL of freshly prepared DPPH solution (0.1 mM) were added to 1 mL of oil and ethyl acetate solution at different concentrations (0.05–25 mg/mL). After 30 min of incubation at 25 °C in the dark, the absorbance of the final solution was measured at 515 nm with Lambda 25 UV/Vis spectrophotometer (PerkinElmer). The percentage of inhibition was calculated using the following equation:

$$\text{Inhibition}=\left(\frac{A_c - A_s}{A_c}\right)\cdot 100 \qquad /2/$$

where A_c and A_s are the absorbance of the control and sample at 515 nm, respectively.

DPPH scavenging activity was expressed as IC_{50}, which indicated the effective sample concentration needed to scavenge 50 % of DPPH radicals and was calculated by a linear regression analysis between the oil concentration and the percentage of inhibition.

Colour measurement

Colour measurements were performed using a HunterLab ColorFlex model A60-1010-615 colourimeter (Reston, VA, USA). Colour values were reported as L* (lightness), a* (redness) and b* (yellowness) according to the Hunter colour scale.

Scanning electron microscopy analysis

Untreated pomagranate seed and solid residues after conventional and microwave-assisted extraction were examined using scanning electron microscopy (SEM) to analyse the effect of extraction methods on the surface morphology of the seeds. Gold/palladium was used for the coating of the samples in an SC7620 sputter coater (Emitech, Kent, UK). Images of samples were taken with JSM-6390LV (JEOL Ltd., Tokyo, Japan) scanning electron

microscope. The SEM images were obtained at 12.5 kV under high vacuum condition and 1000× magnification.

Statistical analysis

The independent *t*-test was used to evaluate differences in the properties of the oil samples obtained by microwave-assisted and cold solvent extraction. The data were analysed using the SPSS v. 22.0 statistical software (SPSS Inc., Chicago, IL, USA).

Results and Discussion

Comparison of extraction efficiencies

Microwave-assisted solvent extraction gave a higher extraction yield of 35.10 % in 5 min than those of Soxhlet extraction (34.70 % in 8 h) and cold solvent extraction (17.50 % in 8 h). Orak *et al*. (*15*) and Özgul-Yücel (*16*) found that the oil content of seeds from different genotypes of *Punica granatum* L. was between 17.84 and 24.96 % (on dry mass basis), which is lower than our results. The differences in the yields might be a result of the genetic backgrounds and the growth conditions of the pomegranate or the applied extraction methods. Taghvaei *et al*. (*6*) reported that the effectiveness of microwaves on rapid extraction of oil from oilseeds was related to the destruction of oil cell structures within the plant tissues through denaturation of cell proteins by heat generated from the movement of polar molecules including water.

Effects of microwave extraction parameters

The effects of different parameters on the MASE of oil from pomegranate seeds were analysed. The experimental and predicted values of the oil extraction yield at each of the 30 runs given by response surface methodology (RSM) are shown in Table 1.

Particle size

Particle size was the most important factor that affected the oil yield in MASE (Table 2). Negative coefficient of the term stated that decreasing the particle size increased the oil extraction yield. The smaller particle size might increase the penetration of irradiation into the interior cell walls and the mass transfer surface area by association of the seed matrix and hexane enhancing the oil extraction. Kwon *et al*. (*31*) and Singh *et al*. (*32*) also documented that there was an inverse relationship between the particle size and microwave extraction yield of different compounds. The effect of interaction between particle size and time on oil extraction yield was also statistically important (Table 2). Fig. 1a illustrates the interaction effect of the particle size and time on extraction yield at a constant solvent-to-sample ratio of 6:1 (by mass) and power of 238 W. The yield at the end of 5-minute extraction was significantly higher (32 %) when using fine particles than when coarse particles were used (11 %) under the same extraction conditions. The yields were 34 and 11 % at the end of 20 min of extraction when fine and coarse particles were used, respectively. This inverse effect might be explained with limited penetration of microwave irradiation into the coarse seeds. It is basically because of zero dielectric constant of cellulose, which is the main component of the seed. Thus, extraction yield could not be improved when using larger particles even though the time increased. Quadratic term of particle size was negative, indicating the presence of maximum value for this variable (Table 2).

Table 2. ANOVA and model equation for response surface quadratic model of microwave- assisted oil extraction

Source	Coefficient	F value	Prob>F p-value
Model*		762.29	<0.0001
Intercept	25.44		
Linear			
t	0.34	4.27	0.0507[a]
ζ(solvent, sample)	2.91	317.05	<0.0001[b]
d(particle)	–11.04	4564.10	<0.0001[b]
Interactive			
t×*d* (particle)	–0.39	5.00	0.0358[b]
ζ(solvent, sample)×*d*(particle)	–1.71	96.78	<0.0001[b]
Quadratic			
ζ(solvent, sample)×ζ(solvent, sample)	–1.90	25.95	<0.0001[b]
d(particle)×*d*(particle)	–3.27	76.54	<0.0001[b]
Lack of fit		1.56	0.3290

*The coefficient of determination (R^2) of the model was 0.99; [a]not significant at 'Prob>F'>0.05, [b]significant at 'Prob>F'<0.05

Solvent-to-sample ratio

Statistical results showed that the solvent-to-sample ratio affected extraction yield significantly (Table 2). The solvent-to-sample ratio had statistically significant interaction effect with particle size. When fine particles of pomegranate seed were used in the microwave extraction, increasing solvent-to-sample ratio from 2:1 to 10:1 (by mass) caused an increase in the extraction yield from 29 to 36 %. However, when the extraction was done with coarse particles of pomegranate seed, increasing solvent--to-sample ratio from 2:1 to 10:1 (by mass) increased the extraction yield from 8 to 12 % (Fig. 1b). This might be explained again by the limited penetration of microwaves into the interior of the coarse seeds. Because of the limited penetration, all of the oil could not be released from the seeds. Hence, there was no extra oil to be dissolved in the medium even though the amount of solvent increased. Quadratic term of solvent-to-sample ratio was found to be statistically significant (Table 2).

Time

The linear term of extraction time was statistically ineffective on oil extraction yield (Table 2). Nde *et al*. (*33*) also reported that time was statistically inefficient on the microwave extraction of neem oil. However, its effect can be understood more obviously by considering the principle of closed-vessel high-pressure microwave extraction. In the present study, closed system microwave application resulted in high pressure extraction of the oil. The combination of the microwaves and high pressure resulted in higher extraction yields at short extraction times

than when using domestic or focused microwave ovens. Optimum extraction times found by Jiao *et al*. (*24*) and Gai *et al*. (*34*) were 66 and 83 min, respectively, which was longer than the value found in this study. In Figs. 1c and d, it can also be seen that increasing the extraction time increased the oil extraction yield. However, these improvements were not statistically significant (Table 2). Similar to this result, Wang *et al*. (*35*) also found that the increase in the extraction yield was very small as a function of time in microwave extraction. The reason for the insignificant increase could be explained by the fact that MASE of different compounds is usually completed within a few minutes to half an hour, depending on the extracted material as mentioned in literature (*33*,*36*–*38*).

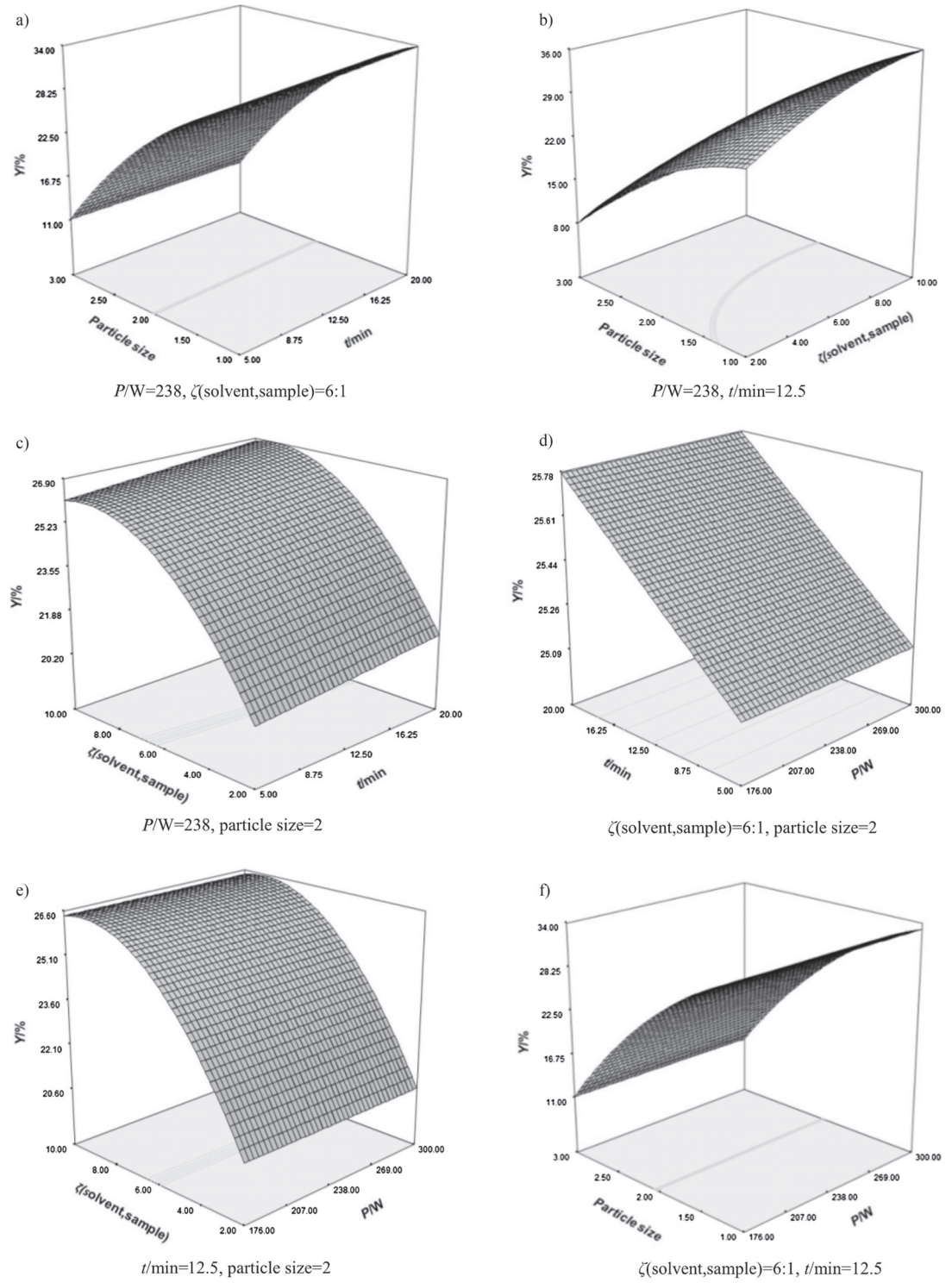

Fig. 1. Response surface plots for oil extraction yield as a function of: a) particle size and time, b) particle size and solvent/sample ratio, c) solvent/sample ratio and time, d) time and power, e) solvent/sample ratio and power, and f) particle size and power. Particle size: 1 (d_1=0.125–0.450 mm), 2 (d_2=0.450–0.530 mm) and 3 (d_3=0.530–0.800 mm)

Power

The linear term of power was omitted by backward elimination to conserve the hierarchy of the model since it had statistically insignificant effect on the oil extraction yield. Figs. 1d, e and f show that increasing the power from 176 to 300 W did not improve the oil extraction yield. The reason for this result might be related to the limited increase in extraction temperature with increasing power. It is well known that higher temperatures increase the oil extraction efficiency due to the higher solubility of oil at higher temperatures. Hexane, which is a microwave-transparent solvent and has low dielectric constant, cannot be heated up to temperature higher than 55 °C during extraction. Thus, hexane might limit the capacity of power to increase extraction efficiency.

Optimisation and validation of extraction conditions

Extraction conditions were optimised for the highest oil extraction yield using Design Expert v. 7.0 software (Stat-Ease, Inc.). The predicted oil extraction yield was 35.19 % under the optimum conditions of 220 W, 5 min, solvent/sample ratio 10:1 (by mass) and d_1=0.125–0.450 mm (fine particles, size 1). Three experiments were performed at these optimum conditions to validate the predicted result, and average oil extraction yield was 34.91 %. The predicted and actual values were in good agreement.

Physical, chemical and some bioactive properties of the pomegranate seed oil

Fatty acid composition

The pomegranate seed oil samples obtained from microwave-assisted and cold solvent extraction had almost identical fatty acid profiles (Table 3). The total unsaturated fatty acid content of pomegranate seed oil obtained by MASE was 95.57 %. The predominant fatty acid was punicic acid (86.53 %) in the oil extracted using microwaves (Table 3). Oleic, linoleic, palmitic and stearic acids were present in minor amounts at 4.10, 3.84, 2.04 and 1.71 %, respectively. The punicic acid and total unsaturated fatty acid content of the investigated pomegranate seed oil were higher than in the papers of Pereira de Melo *et al.* (*14*), Fadavi *et al.* (*22*) and Khoddami *et al.* (*39*). The dissimilarity might be related to the differences in pomegranate cultivars and climatic conditions during growth.

Table 3. Fatty acid composition of pomegranate seed oil extracted by microwave-assisted and cold solvent extraction

Fatty acid	*w*/%	
	MASEO	CSEO
C16:0	2.04±0.01	2.34±0.01
C17:0	0.04±0.01	0.04±0.01
C18:0	1.71±0.02	1.69±0.01
C18:1	4.10±0.01	4.07±0.01
C18:2	3.84±0.02	3.85±0.02
C18:3	86.53±0.01	86.42±0.02
C20:0	0.37±0.02	0.37±0.01
C20:1	0.69±0.01	0.70±0.01
C20:3	0.09±0.01	0.09±0.01
C20:4	0.32±0.01	0.31±0.01
C24:0	0.27±0.01	0.12±0.02
Total polyunsaturated fatty acids	90.78	90.67
Total monounsaturated fatty acids	4.79	4.77
Total saturated fatty acids	4.43	4.56

All values are expressed as mean±standard deviation of three replicates.
MASEO=microwave-assisted solvent extraction of oil,
CSEO=cold solvent extraction of oil

Peroxide value and acidity

Peroxide value and acidity are the indices for the determination of the oxidative degradation of products in oil. The peroxide value of pomegranate seed oil obtained by cold solvent extraction was 4 mmol of O_2 per kg, while that of the oil extracted by microwave-assisted extraction was 0 mmol of O_2 per kg (Table 4). The long extraction time (8 h) and an extraction process under atmospheric pressure could be the reasons for higher peroxide value of the oil extracted by cold solvent extraction. In the oil extracted by cold solvent and microwave-assisted solvent extractions, there was 0.42 and 0.44 % free fatty acids, expressed as punicic acid, respectively.

Table 4. Physical, chemical and bioactive properties of pomegranate seed oil extracted by microwave-assisted and cold solvent extraction

Property	MASEO	CSEO
Peroxide value/(mmol of O_2 per kg of oil)	0.00±0.05	4.00±0.12
w(FFA as punicic acid)/%	0.42±0.03	0.44±0.02
w(total phenols as GAE)/(mg/g)	7.42±0.12	1.73±0.05
IC_{50}/(mg/mL)	17.00±0.21	5.12±0.22
L*	56.03±0.01	58.91±0.02
a*	–5.64±0.03	–2.45±0.03
b*	22.06±0.02	14.44±0.57

All comparisons of MASEO and CSEO in all analyses were significant, with $p<0.05$.
All values are expressed as mean±standard deviation of three replicates.
MASEO=microwave-assisted solvent extraction of oil,
CSEO=cold solvent extraction of oil, FFA=free fatty acid,
GAE=gallic acid equivalent, IC_{50}=concentration of antioxidant that causes 50 % inhibition of DPPH, L*=lightness, a*=redness, b*=yellowness

Total phenolic content

The total phenolic content expressed in gallic acid equivalents of the pomegranate seed oil obtained by microwave-assisted and cold solvent extraction were 7.42 and 1.73 mg/g, respectively (Table 4). The higher total phenolic content of pomegranate seed oil obtained by MASE can be related to higher pressure during the extraction. The presented extraction system combines the advantage of microwave-assisted and pressurised solvent extraction. Bayramoglu *et al.* (*40*) also reported that higher internal pressure of the solid media and hence enhancement of the extraction may be the reason for higher

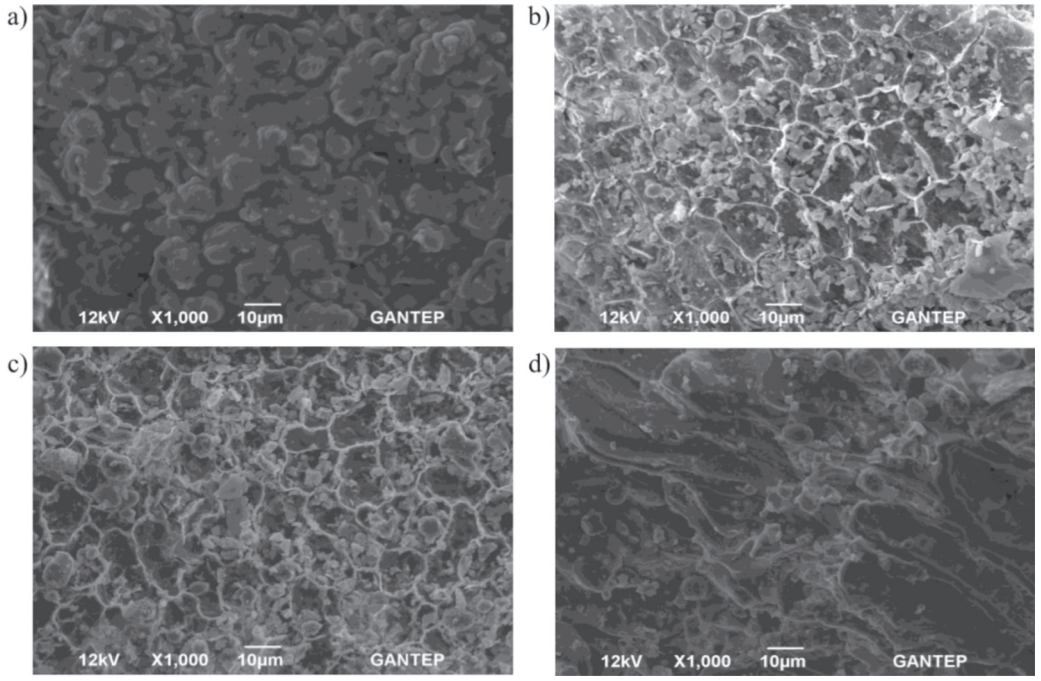

Fig. 2. SEM images of pomegranate seed samples: a) untreated pomegranate seed, b) solid residue after Soxhlet extraction, c) solid residue after cold solvent extraction and d) solid residue after microwave-assisted solvent extraction (MASE). MASE was performed under optimum conditions

phenolic content of the oil obtained by MASE. Similarly, Haddadi-Guemghar *et al.* (*41*) also found that the phenolic content of the oil extracted by conventional method was significantly lower than that of the oil obtained by MASE.

Antioxidant activity

IC_{50} value of the oil extracted by microwave-assisted extraction (5.12 mg/mL) was significantly lower than that of the oil extracted by cold extraction (17.00 mg/mL) (Table 4). This significant difference in the IC_{50} values might be related to the differences in total phenolic content of the extracted oil depending on the extraction method. He *et al*. (*42*) demonstrated a significant relationship between DPPH activities and total phenolics (R^2=0.751) in pomegranate seed residues. Gil *et al*. (*43*) also reported that pomegranate fruit is a rich source of two types of polyphenolic compounds: anthocyanins and hydrolysable tannins, which account for 92 % of the antioxidant activity of the whole fruit. This showed that higher total phenolic content in the oil obtained by microwave-assisted extraction significantly increased the antioxidant activity of the oil when compared to that of the oil obtained by cold solvent extraction.

Colour

The colour values of the oil extracted by two different methods were significantly different (Table 4). The lightness (L*) of pomegranate seed oil extracted by cold extraction method (58.91) was higher than that of the oil extracted by MASE (56.03). The a* value measures redness (+) and greenness (–) and the b* value indicates yellowness (+) and blueness (–). The b* value of the oil extracted by MASE (22.06) was higher than that of the oil extracted by cold extraction (14.44), while a* value of the oil extracted by MASE (–5.64) was lower than that of the oil extracted by cold solvent extraction (–2.45). These results showed that MASE was more efficient in extracting the chlorophyll and carotene present in the pomegranate seeds.

Structural changes of pomegranate seeds

SEM analyses were performed to observe the microscopic changes in pomegranate seed before and after extraction to compare the influence of conventional and microwave-assisted solvent extractions on the pomegranate seed structure. The fat globules were dispersed uniformly in the tissues of pomegranate seed before the extraction (Fig. 2a). On the SEM images of the residues after Soxhlet and cold solvent extraction, the fat globules disappeared while the cell structure was preserved and the cells were still entirely unbroken (Figs. 2b and c). However, MASE caused some structural changes in the seed tissues (Fig. 2d). Most of the cell walls and membranes of the pomegranate seeds were ruptured and broken down completely after MASE (Fig. 2d). It is clearly seen that the oil can be released from the cell structure and extracted efficiently by cell wall rupture in a short time. There was a good correspondence between these results and the findings of Jiao *et al*. (*24*), who studied the Soxhlet and MASE of pumpkin seeds.

Conclusion

Closed-vessel high-pressure microwave system was optimised to obtain the highest performance in extraction of pomegranate seed oil. Verification experiments resulted in the extraction yield of 34.91 % under the optimum extraction conditions and are in good agreement with the

predicted value of 35.19 %. The oil extraction yield obtained by MASE was higher than those obtained by conventional extractions. The fatty acid compositions of the oil extracted by cold and microwave-assisted solvent extractions were statistically similar to each other ($p<0.05$). Oil extracted by MASE had significantly lower peroxide value (0 mmol of O_2 per kg of oil), free fatty acidity (0.42 %) and higher total phenolic content expressed in gallic acid equivalents (7.42 mg/g) and antioxidant activity (5 mg/mL) than those of the oil obtained by cold extraction. SEM results demonstrated that microwave method broke down cell walls and membranes efficiently, thus increasing the efficient release of oil from the seed in shorter time than in conventional extraction. The results indicated that higher quality oil could be obtained using MASE than when using cold extraction. The application of high-efficiency short-time MASE can be a valuable alternative to conventional oil extraction methods, especially for healthcare, pharmaceutical and healthy food industries.

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