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ORIGINAL PAPER

Extraction of Essential Oils From the Seeds of Pomegranate Using Organic Solvents and Supercritical CO_2

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Abstract In this study, essential oils from pomegranate seeds of the Malas variety from Shahreza, Iran, were extracted using hexane and petroleum benzene applying four extraction methods: normal stirring, soxhlet, microwave irradiation, and ultrasonic irradiation. Also, supercritical fluid extraction (SFE) using CO_2 under different conditions was used for comparison. Different methods of extraction with organic solvents (normal stirring, soxhlet, microwave irradiation, and ultrasonic irradiation) showed significant differences in the extraction yields. However, no differences were found when a given method (e.g., microwave irradiation) was applied using different organic solvents. On the other hand, different extraction conditions from the various runs of SFE resulted in different extraction yields, all of which were lower than those of the other extraction methods using organic solvents. No significant differences were observed in the fatty acid compositions of the extracted oils using organic solvents. However, the fatty acid compositions of the oils extracted under different conditions of the SFE system indicated significant differences among several fatty acids including unsaturated fatty acids.

Keywords Essential oils · Fatty acid composition · Pomegranate seed oil · Punicic acid · Supercritical fluid extraction

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Introduction

Pomegranate (*Punica granatum L.*) is one of the oldest edible fruit and belongs to the Punicacea family [1]. Pomegranate is extensively cultivated in the Mediterranean area and most Near- and Far East countries [2]. Iran is a native land of pomegranate. The total pomegranate production in Iran was reported at 665,000 tons for the year 2003 [1]. The edible part of pomegranate is called aril and constitutes about 52% (w/w) of the total fruit and comprises of 78% juice and 22% seeds [3]. Pomegranate seeds contain high amounts of oil, in which in some Iranian varieties the total lipid content on a dry basis ranged from 66 to 193 g in one kg of the fruit [1].

Pomegranate seed oil consists of 65–80% conjugated fatty acids, the most important of which is 9-*trans*, 11-*cis*, 13-*trans*, octadecatrienoic acid, the so-called punicic acid. Pomegranate seed oil was reported to inhibit the upstream eicosanoid enzyme phospholipase A_2 expressed by human prostate cancer cells [4]. In addition, pomegranate seed oil can decrease the leukotriene production from arachidonic acid, which plays a major role in the occurrence of asthma in children, skin inflammation and platelet aggregation associated with cardiovascular diseases [2]. The objectives of this work were to extract the essential oils from pomegranate seeds using various extraction methods and to determine the fatty acid compositions of the extracted oils.

Materials and Methods

According to a previous report, "sour–sweet" varieties of pomegranate seeds contain more oil than do other varieties [1]. Therefore, the pomegranate selected for this study was from the Malas variety obtained from Shahreza, in the

Isfahan province of Iran. After transferring the fruit to the laboratory, those with the defective areas (i.e., those with sunburns, cracks, and bruises in the husk) were discarded. Then, seeds from the rest of the fruit were separated from the juice and washed carefully to remove sugars and other adhering materials. Separated seeds were dried at 35–37 °C until a constant weight was reached. Dried pomegranate seeds were pulverized and particles with size distribution of less than 40-mesh was used for the extraction.

Extraction of Seed Oils

Soxhlet Extraction

Five grams of the crushed dry seeds were refluxed using two different organic solvents (petroleum benzene and hexane: 155 mL) in a Soxhlet apparatus. The solvents were then evaporated in a vacuum oven at 35 °C and the extracts were dried until a constant weight using a mild flow of nitrogen gas.

Normal Stirring Extraction

A five-gram sample was blended with 20 mL solvent (petroleum benzene and hexane) and mixed thoroughly using a magnetic stirrer for 4 h. Then, the solvent was evaporated in a vacuum oven as explained above.

Sonication and Extraction Procedure

Five grams of ground pomegranate seeds was mixed with 20 mL solvent. The sample–solvent suspension was then ultrasonicated for 45 min using an ultrasonic bath at ambient conditions. Extracted oil was separated applying the same method as explained above.

Microwave-assisted Extraction

Five grams of crushed pomegranate seeds was mixed with 20 mL solvent. The suspension was then irradiated in a household microwave oven (full power = 1,000 W, Butane, Iran) for 30 s at different power levels (200, 400, 600, 800 W), and then the suspension was cooled to ambient conditions. This cycle was continued for a total heating time of 10 min, when oil extraction was completed and more heating time did not affect the extraction yield. Oil separation was then carried out applying the procedure explained under "Soxhlet Extraction" [5].

Supercritical Fluid Extraction (SFE) of Seed Oil

A Suprex MPS/225 system (Pittsburgh, PA) in the SFE mode was used for all extractions with supercritical CO_2. The extraction chamber was a 10-mL stainless steel cell and a Duraflow manual variable restrictor (Suprex) was used for collecting the extracted analytes. To prevent sample precipitation at this point the restrictor was heated using an electrical heat jacket. A volumetric flask containing 5.0 mL dichloromethane placed in an ice-bath was used to improve the sample recovery from the restrictor. To carry out the extractions, a 5.6-g sample was mixed with 2 mm-diameter glass beads and charged into the extraction cell. Each of the experimental parameters (pressure, temperature, kind of modifier and amount of modifier) at three levels (Table 1) were applied according to the Taguchi's statistical design procedure [6].

Fatty Acid Composition

Fatty acid methyl ester preparation was the first stage to determine fatty acid (FA) composition of the extracted oils. For this purpose, sample was mixed vigorously with

Table 1 Various experimental conditions applied in the extraction of pomegranate seed oil using supercritical fluid CO_2 along with the yields of the extracted oils

Run no	Pressure (atm)	Temperature (°C)	Modifier	Modifier volume (mL/100 g)	Yield (%, w/w)
1	200	40	Water	0	0.89
2	200	50	Ethanol	9	1.34
3	200	60	Hexane	18	2.86
4	275	40	Ethanol	18	3.39
5	275	50	Hexane	0	1.88
6	275	60	Water	9	0.80
7	350	40	Hexane	9	3.21
8	350	50	Water	18	2.50
9	350	60	Ethanol	0	2.14

0.1 mL alcoholic potassium hydroxide (11.2 g KOH in 100 mL pure methanol). After 20 s, 1 mL hexane was added to the suspension and was held in water bath at 50 °C for 15 min [7]. Finally, the transparent upper layer of the suspension, which included methyl esters, was used for the analysis by GC. A Varian Star 3400 gas chromatograph equipped with a capillary column (DB-23, J & W Scientific, 30 m × 0.25 mm i.d. × 0.25 μm thick) was used for this purpose. The detector (FID) and injector temperatures were set at 250 and 220 °C, respectively. The initial oven temperature was 190 °C and was increased to 220 °C at a rate of 1 °C/min. The peaks were identified by comparing their retention times with those of an authentic standard fatty acid methyl ester mixture [8].

Statistical Data Analysis

One-factorial data analysis to compare the means among the different treatments was performed using MSTAT-C statistical software. To compare the data between the solvents and among the various extraction methods, two-factorial data analysis using the same software was applied.

Results and Discussion

Extraction Yield

The yields of the extracted oils from various conditions of supercritical fluid extraction are shown in Table 1. Means of the extraction yields for different operational conditions were determined following Taguchi's statistical approach [6]. Figure 1 shows the changes in the extraction yield with a change in pressure, temperature, type of modifier and modifier volume. The extraction yield increased with an increase in pressure due to an increase in the fluid density and therefore the solvent power of CO_2. However, due to the adverse effect of temperature on the solvent power, raising the temperature resulted in a slight decrease in the extraction yield. Application of water, ethanol and hexane as modifiers resulted in an increase in the extraction yield in the order hexane > ethanol > water. Such results can be attributed to the decrease in the solvent polarity favoring the extraction of less polar components of seed oil. Furthermore, increasing the volume of the modifier showed a positive effect on the extraction yield. Among the parameters studied, modifier volume showed the greatest influence on the extraction yield (Table 2) with temperature having the least effect.

The yields of extractions applying various methods of this study are shown in Table 3. No significant differences were observed between the extraction yields when using

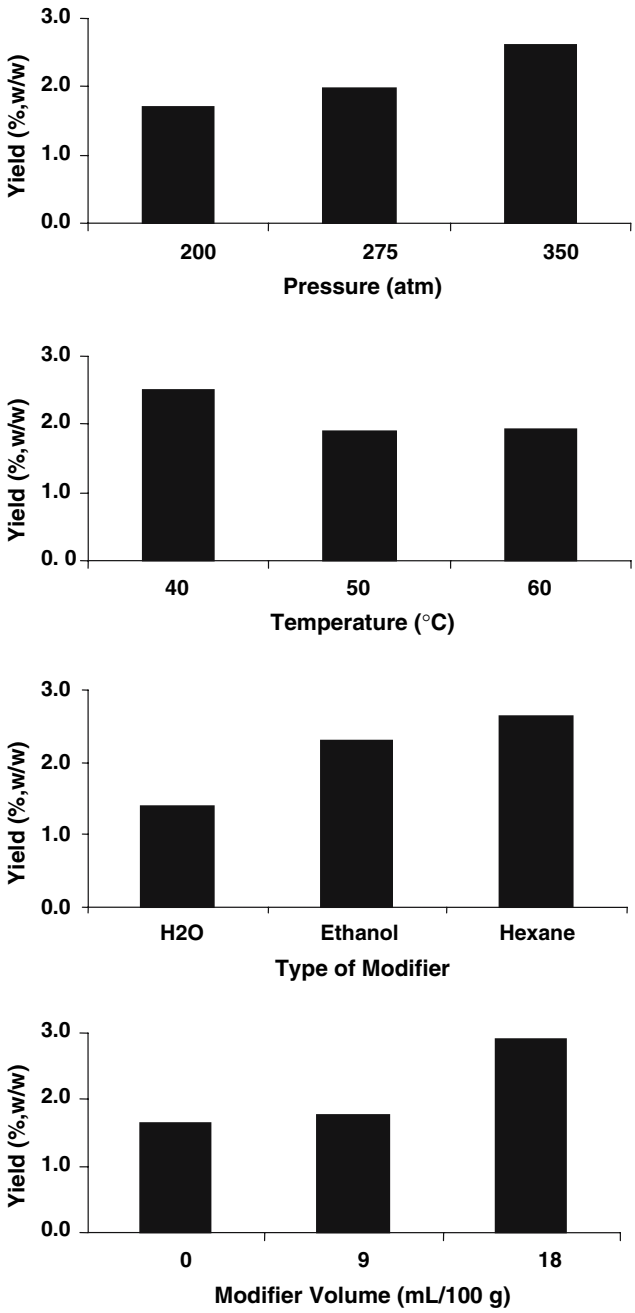

Fig. 1 Effect of pressure, temperature, type of modifier and modifier volume on the yield of extracted oils in SFE system

the two applied solvents, petroleum benzene and hexane, but various extraction methods indicated significant differences. Comparing the extraction yield from the stirred samples with that of the blank indicated that increasing contact time between solvent and sample did not have any significant effect on the yield of the extraction in this case but ultrasonic irradiation by creating microstructure fracture on the cell wall and microwave irradiation (by increasing the solvent temperature and improving its penetration power as well as the diffusion coefficients of the

Table 2 Contribution of different parameters on the extraction yield

Parameter	*d*	*S*	*V*	*P*	*S*′	*P* (%)
Pressure	2	1.31	0.66	1.90	0.62	17.7
Temperature	2	0.68	0.34			9.22
Type of modifier	2	2.48	1.24	3.60	1.79	33.5
Modifier volume	2	2.93	1.47	4.25	2.24	39.5
Error	6	0.69				
Total	14	8.10				100

d Degree of freedom, *S* mean square, *V* variance, *F* V/V_e, *S*′ pure mean square, *P* S/S_{tot}

Table 3 Total oil recovery applying different extraction methods using petroleum benzene and hexane as solvents (Micro stands for microwave)

Extraction method	Solvent	
	Petroleum benzene	Hexane
Blank	13.0 ± 0.0^{e}	12.7 ± 0.4^{e}
Stirring (4 h)	13.0 ± 0.0^{e}	13.0 ± 0.3^{e}
Ultrasound (45 min)	15.7 ± 0.1^{bc}	16.0 ± 0.5^{b}
Micro—200 W (10 min)	14.7 ± 0.4^{b}	15.0 ± 0.0^{cd}
Micro—800 W (10 min)	15.6 ± 0.3^{bcd}	15.8 ± 0.3^{bc}
Soxhlet (6 h)	18.6 ± 0.2^{a}	18.7 ± 0.2^{a}

Two-factorial comparison among the data. Means with the same letters are not significantly different ($p < 0.01$)

solutes) resulted in higher extraction yield. Soxhlet extraction led to the highest extraction level due to use of larger amount of solvent (155 mL) resulting in a greater solvent/sample ratio in the soxhlet extraction method compared to the other extraction methods studied here. The simplicity of the new methods could compensate for the longer time and larger solvent volumes used in the traditional extraction methods without any adverse effects on the extraction yield and the quality of the extract.

Fatty Acid Composition

Effects of solvent type and extraction method on the fatty acid compositions of the extracted oils are shown in Tables 4, 5 and 6. Twelve fatty acids ($C_{16:0}$, $C_{18:0}$, $C_{18:1}$, $C_{18:2}$, $C_{18:3}$, $C_{18:3}$ (punicic), $C_{20:0}$, $C_{20:1}$, $C_{22:0}$, $C_{22:1}$, $C_{24:0}$ and $C_{24:1}$) at different quantities were detected in all samples. Punicic, linoleic, oleic, palmitic and stearic acids were among the highest with the following order: punicic > linoleic > oleic > palmitic > stearic. No significant differences were observed in the fatty acid compositions of the extracted oils using organic solvents in various extraction methods using ultrasound and microwave irradiations,

Table 4 Fatty acid compositions (mean ± SD) of the extracted oils using SFE at different operational conditions

Run no.	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	$C_{18:3}$ (punicic)	$C_{20:0}$	$C_{20:1}$	C_{22}	$C_{24:0}$	$C_{22:1}$	$C_{24:1}$
1	3.9 ± 0.4^{ab}	2.6 ± 0.1^{a}	8.6 ± 1.9^{ab}	10.8 ± 0.2^{a}	0.4 ± 0.1^{a}	71.0 ± 0.3^{d}	0.4 ± 0.1^{a}	0.4 ± 0.1^{a}	0.2 ± 0.1^{a}	1.9 ± 0.2^{a}	ND^{a}	ND^{a}
2	4.6 ± 0.5^{a}	2.7 ± 0.0^{a}	9.3 ± 0.8^{a}	10.8 ± 0.1^{a}	ND^{b}	69.8 ± 0.9^{d}	0.4 ± 0.1^{a}	0.4 ± 0.0^{a}	0.2 ± 0.2^{a}	1.8 ± 0.2^{a}	ND^{a}	ND^{a}
3	2.9 ± 0.0^{c}	2.2 ± 0.1^{a}	7.1 ± 0.1^{c}	7.3 ± 0.0^{d}	ND^{b}	79.0 ± 0.2^{a}	0.4 ± 0.0^{a}	0.5 ± 0.0^{a}	0.2 ± 0.1^{a}	0.4 ± 0.0^{d}	ND^{a}	0.1 ± 0.0^{a}
4	3.2 ± 0.2^{a}	2.2 ± 0.2^{bc}	7.6 ± 0.5^{bc}	8.8 ± 0.6^{c}	ND^{b}	76.9 ± 1.6^{ab}	0.4 ± 0.0^{a}	0.5 ± 0.0^{a}	ND^{a}	0.5 ± 0.1^{d}	ND^{a}	ND^{a}
5	3.4 ± 0.0^{bc}	2.4 ± 0.0^{a}	7.9 ± 0.3^{bc}	9.6 ± 0.3^{bc}	ND^{b}	74.4 ± 1.1^{bc}	0.4 ± 0.0^{a}	0.5 ± 0.0^{a}	ND^{a}	1.3 ± 0.5^{abc}	ND^{a}	ND^{a}
6	4.1 ± 0.1^{a}	3.1 ± 0.7^{a}	8.4 ± 0.1^{ab}	10.2 ± 0.0^{ab}	ND^{b}	71.9 ± 1.0^{cd}	0.4 ± 0.0^{a}	0.5 ± 0.0^{a}	ND^{a}	1.5 ± 0.3^{ab}	ND^{a}	ND^{a}
7	3.4 ± 0.0^{bc}	2.3 ± 0.1^{a}	7.8 ± 0.1^{bc}	8.6 ± 0.3^{c}	ND^{b}	76.3 ± 0.7^{ab}	0.4 ± 0.0^{a}	0.5 ± 0.0^{a}	0.2 ± 0.1^{a}	0.6 ± 0.1^{cd}	ND^{a}	ND^{a}
8	3.4 ± 0.0^{bc}	2.4 ± 0.0^{a}	8.0 ± 0.1^{bc}	9.0 ± 0.1^{c}	ND^{b}	75.6 ± 0.5^{b}	0.4 ± 0.0^{a}	0.5 ± 0.0^{a}	0.1 ± 0.1^{a}	0.6 ± 0.0^{cd}	ND^{a}	ND^{a}
9	3.4 ± 0.0^{bc}	2.4 ± 0.2^{a}	7.8 ± 0.2^{bc}	9.4 ± 0.3^{bc}	ND^{b}	75.3 ± 0.7^{b}	0.4 ± 0.0^{a}	0.5 ± 0.0^{a}	ND^{a}	0.9 ± 0.2^{bcd}	ND^{a}	ND^{a}

Means with the same letters are not significantly different ($p < 0.01$)

ND Not detected

Table 5 Fatty acid compositions of the extracted oils in different extraction systems using petroleum benzene as solvent (Micro stands for microwave)

Sample	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	$C_{18:3}$ (punicic)	$C_{20:0}$	$C_{20:1}$	$C_{22:0}$	$C_{22:1}$	$C_{24:0}$	$C_{24:1}$
Blank	2.4 ± 0.3^a	2.3 ± 0.4^a	6.2 ± 0.4^a	6.1 ± 0.1^a	ND[a]	81.8 ± 0.3^a	0.4 ± 0.0^a	0.5 ± 0.0^a	0.2 ± 0.0^a	ND[a]	0.1 ± 0.0^a	0.1 ± 0.1^a
Stirring	2.2 ± 0.2^a	1.9 ± 0.1^a	6.4 ± 0.3^a	6.1 ± 0.2^a	ND[a]	82.1 ± 0.6^a	0.4 ± 0.0^a	0.5 ± 0.0^a	0.1 ± 0.1^a	ND[a]	0.1 ± 0.0^a	0.1 ± 0.0^a
Ultrasound	2.2 ± 0.2^a	2.0 ± 0.0^a	6.4 ± 0.2^a	6.6 ± 0.1^a	ND[a]	81.7 ± 0.2^a	0.4 ± 0.1^a	0.6 ± 0.1^a	0.1 ± 0.1^a	ND[a]	ND[a]	ND[a]
Micro—200 W (5 min)	2.5 ± 0.0^a	2.0 ± 0.5^a	6.8 ± 0.0^a	6.2 ± 0.1^a	ND[a]	81.0 ± 0.6^a	0.4 ± 0.0^a	0.5 ± 0.1^a	0.2 ± 0.1^a	ND[a]	0.1 ± 0.0^a	0.1 ± 0.0^a
Micro—200 W (10 min)	2.3 ± 0.0^a	2.1 ± 0.0^a	6.5 ± 0.1^a	6.2 ± 0.2^a	ND[a]	81.7 ± 0.3^a	0.4 ± 0.0^a	0.5 ± 0.1^a	0.1 ± 0.0^a	ND[a]	0.1 ± 0.0^a	0.1 ± 0.0^a
Micro—800 W (5 min)	2.4 ± 0.1^a	1.7 ± 0.4^a	6.8 ± 0.1^a	6.2 ± 0.1^a	ND[a]	81.5 ± 0.4^a	0.4 ± 0.1^a	0.5 ± 0.0^a	0.1 ± 0.0^a	ND[a]	0.1 ± 0.0^a	0.2 ± 0.0^a
Micro—800 W (10 min)	2.0 ± 0.3^a	1.9 ± 0.3^a	6.0 ± 0.1^a	6.1 ± 0.2^a	ND[a]	82.9 ± 0.4^a	0.4 ± 0.0^a	0.5 ± 0.1^a	0.2 ± 0.3^a	ND[a]	0.1 ± 0.0^a	0.1 ± 0.1^a
Soxhlet	2.4 ± 0.2^a	2.0 ± 0.2^a	6.6 ± 0.1^a	6.6 ± 0.2^a	ND[a]	81.5 ± 0.7^a	0.2 ± 0.2^a	0.4 ± 0.2^a	0.2 ± 0.3^a	ND[a]	0.1 ± 0.0^a	ND[a]

Means with the same letters are not significantly different ($p < 0.01$)

ND Not detected

Table 6 Fatty acid compositions of the extracted oils in different extraction systems using hexane as solvent (Micro stands for microwave)

Sample	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	$C_{18:3}$ (punicic)	$C_{20:0}$	$C_{20:1}$	$C_{22:0}$	$C_{22:1}$	$C_{24:0}$	$C_{24:1}$
Blank	2.2 ± 0.2^a	2.1 ± 0.1^a	6.4 ± 0.1^a	6.4 ± 0.3^a	ND[a]	81.7 ± 0.3^a	0.4 ± 0.1^a	0.6 ± 0.0^a	0.1 ± 0.1^a	ND[a]	0.1 ± 0.0^a	ND[a]
Stirring	2.3 ± 0.1^a	2.1 ± 0.0^a	6.5 ± 0.1^a	6.4 ± 0.1^a	ND[a]	81.5 ± 0.0^a	0.4 ± 0.0^a	0.5 ± 0.0^a	0.1 ± 0.0^a	ND[a]	0.1 ± 0.0^a	ND[a]
Ultrasound	2.3 ± 0.0^a	1.9 ± 0.0^a	6.3 ± 0.4^a	6.3 ± 0.5^a	ND[a]	82.0 ± 0.6^a	0.4 ± 0.1^a	0.5 ± 0.0^a	0.1 ± 0.1^a	ND[a]	0.1 ± 0.0^a	0.1 ± 0.1^a
Micro—200 W (5 min)	2.0 ± 0.3^a	1.9 ± 0.1^a	5.9 ± 0.4^a	6.0 ± 0.3^a	ND[a]	83.1 ± 0.5^a	0.4 ± 0.0^a	0.5 ± 0.0^a	0.1 ± 0.0^a	ND[a]	0.1 ± 0.0^a	ND[a]
Micro—200 W (10 min)	2.4 ± 0.5^a	2.0 ± 0.2^a	6.6 ± 0.6^a	6.7 ± 0.0^a	ND[a]	81.1 ± 1.3^a	0.4 ± 0.0^a	0.5 ± 0.0^a	0.1 ± 0.0^a	0.1 ± 0.03^a	0.1 ± 0.0^a	ND[a]
Micro—800 W (5 min)	2.2 ± 0.1^a	2.0 ± 0.0^a	6.5 ± 0.1^a	6.4 ± 0.2^a	ND[a]	81.7 ± 0.1^a	0.4 ± 0.0^a	0.5 ± 0.0^a	0.1 ± 0.0^a	ND[a]	0.1 ± 0.0^a	0.1 ± 0.0^a
Micro—800 W (10 min)	2.4 ± 0.3^a	1.8 ± 0.2^a	6.4 ± 0.2^a	6.1 ± 0.2^a	ND[a]	82.2 ± 0.9^a	0.3 ± 0.12^a	0.4 ± 0.1^a	0.2 ± 0.0^a	ND[a]	0.1 ± 0.0^a	ND[a]
Soxhlet	2.5 ± 0.1^a	2.1 ± 0.1^a	6.6 ± 0.2^a	6.3 ± 0.4^a	ND[a]	81.4 ± 0.7^a	0.4 ± 0.0^a	0.4 ± 0.1^a	0.2 ± 0.0^a	ND[a]	0.1 ± 0.0^a	[a]ND

Means with the same letters are not significantly different ($p < 0.01$)

ND Not detected

stirring or heating with circulation. However, the fatty acid compositions of the oils extracted under the different conditions of SFE system indicated significant differences in $C_{16:0}$, $C_{18:0}$, $C_{18:1}$, $C_{18:2}$, $C_{18:3}$ and $C_{24:0}$ levels but no significant differences were observed in the levels of other fatty acids. $C_{18:3}$ in the form of punicic acid was the major fatty acid found among all samples, which was in agreement with the results of Fadavi et al. [1]. Fadavi et al. [1] determined the fatty acid compositions of the seeds from 25 Iranian pomegranates varieties. Total $C_{18:3}$ FA concentration of over 80% in pomegranate seed oil is the highest possible among many oils reported in literature. Some vegetable oils such as those from palm, palm kernel, coconut, cocoa butter, olive, hazelnut, safflower, cottonseed, sunflower and corn contain somewhere between 0.0 and 1.0% $C_{18:3}$ and oils from soybean and rapeseed contain 7.8 and 9.9% $C_{18:3}$, respectively [9]. Instead, these oils contain relatively high levels of $C_{18:2}$ FA. Among the vegetable oils mentioned here, safflower oil at 76% $C_{18:2}$ is a good example. Furthermore, walnut oil with 74.0% $C_{18:2}$ and 10.0% $C_{18:3}$ has an overall polyunsaturated fatty acids (PUFA) content of over 84% [9]. Similarly, raspberry seed oil with 54.5% $C_{18:2}$ and 29.1% $C_{18:3}$ and evening primrose oil with 73.8% $C_{18:2}$ and 9.6% $C_{18:3}$ have close to 84% PUFA. Some vegetable oils with $C_{18:3}$ content as high as 61.3% was reported by Dubois et al. [9].

The presence of $C_{18:2}$ in the extracted oils from pomegranate seeds from this study was in agreement with the results of Melgarejo and Artes [10]. However, caproic, capric and myristoleic acids that were previously reported by El-Nemr et al. [11] were not found in this study. El-Nemr et al. [11] reported caprylic and stearic acids as the major fatty acids in a sweet Egyptian pomegranate cultivar. Considering $C_{18:2}$ content of over 6.0% in pomegranate seed oil, its total PUFA content exceeds 88.0%, which makes this oil extraordinarily important considering the numerous health benefits associated with PUFA.

The ratios of saturated/unsaturated (*S*/*U*) fatty acids were also determined among all the oils extracted at the various conditions of this study. In the extracted oils from SFE system, the lowest *S*/*U* ratio was at 0.06 level (run no. 3) and the highest was at 0.11 level (run no. 2) (Table 7). These ratios were in agreement with the previous results that the *S*/*U* fatty acid ratios of 25 Iranian pomegranate cultivars were reported in 0.05–0.37 range [1]. Total amount of unsaturated fatty acids (UFA) was also increased with an increase in pressure. Among different types of modifiers studied here, hexane was the most effective in the extraction of UFA. Also, the use of modifier at its highest level resulted in the greatest level of UFA extraction (93.9 ± 0.1, according to their respective peak areas). The effect of temperature on the extraction of UFA was minimal and only 9.63% of total changes in the amount of UFA were related to the change of temperature (Table 8). As punicic acid was the major UFA, different parameters also indicated a direct effect on the level of punicic acid. Run number 3 (pressure = 350 atm, temperature = 60 °C, modifier = hexane at its maximum level studied) resulted in the greatest level of punicic acid extraction. Decreasing the polarity of SFE system with the addition of hexane as a modifier resulted in an increase in the extraction of punicic acid and in turn that of UFA.

Table 7 Concentrations of unsaturated fatty acids (UFA) and ratio of saturated/unsaturated fatty acids (*S*/*U*) of the extracted oils using SFE at different operational conditions

Run no.	UFA	*S*/*U*
1	91.3 ± 0.0^{cd}	0.10 ± 0.00^{ab}
2	90.3 ± 0.4^{d}	0.11 ± 0.01^{a}
3	93.9 ± 0.1^{a}	0.06 ± 0.00^{c}
4	93.7 ± 0.5^{ab}	0.07 ± 0.01^{c}
5	92.4 ± 0.5^{bc}	0.08 ± 0.01^{bc}
6	91.0 ± 0.9^{cd}	0.10 ± 0.01^{ab}
7	93.2 ± 0.3^{ab}	0.07 ± 0.00^{c}
8	93.0 ± 0.3^{ab}	0.08 ± 0.00^{c}
9	93.0 ± 0.3^{ab}	0.08 ± 0.00^{c}

Mean ± SD. In each column, means with the same letters are not significantly different ($p < 0.01$)

Linoleic acid was the second most abundant fatty acid found in the extracted oil using SFE system, which ranged between 7.3 and 10.8% (w/w) among different runs. Among all fatty acids, oleic acid changed within 7.1–9.3% range among different runs of SFE. Stearic and palmitic acids were found at noticeably lower levels than did the other fatty acids. Ratios of *S*/*U* fatty acids and total UFA of the extracted oils using the two organic solvents are shown in Table 9. *S*/*U* ratios were at 0.05–0.06 level indicating that UFA has greater contribution in the extracted oils using organic solvents than have the oils extracted by SFE system. Linoleic acid changed between 6.1 and 6.7% (w/w), which was similar to changes happening on oleic acid (5.9–6.8%). This indicates that

Table 8 Contribution of different parameters on the amount of unsaturated fatty acids

Parameter	*d*	*S*	*V*	*F*	*S*′	*P* (%)
Pressure	2	2.30	1.15	1.85	1.05	17.9
Temperature	2	1.24	0.62			9.63
Type of modifier	2	2.98	1.49	2.36	1.73	23.1
Modifier volume	2	6.36	3.20	5.12	5.12	49.4
Error	6	1.25				
Total	14	14.12				100

d Degree of freedom, *S* mean square, *V* variance, *F* V/V_e, *S*′ pure mean square, *P* S/S_{tot}

Table 9 Concentration of unsaturated fatty acids (UFA) and ratio of saturated/unsaturated fatty acids (*S*/*U*) of the extracted oils using petroleum benzene and hexane as solvents applying different extraction systems (Micro stands for microwave)

Extraction method	UFA		*S*/*U*	
	Petroleum benzene	Hexane	Petroleum benzene	Hexane
Blank	94.7 ± 0.6[a]	95.1 ± 0.2[a]	0.06 ± 0.01[a]	0.05 ± 0.00[a]
Stirring (4 h)	95.2 ± 0.5[a]	94.9 ± 0.1[e]	0.05 ± 0.01[a]	0.05 ± 0.00[a]
Ultrasound (45 min)	95.3 ± 0.1[a]	95.3 ± 0.0[a]	0.05 ± 0.00[a]	0.05 ± 0.00[a]
Micro—200 W (5 min)	94.6 ± 0.5[a]	95.5 ± 0.4[a]	0.06 ± 0.01[a]	0.05 ± 0.00[a]
Micro—200 W (10 min)	95.0 ± 0.1[a]	95.0 ± 0.7[a]	0.05 ± 0.00[a]	0.05 ± 0.01[a]
Micro—800 W (5 min)	95.2 ± 0.7[a]	95.1 ± 0.0[a]	0.05 ± 0.00[a]	0.05 ± 0.00[a]
Micro—800 W (10 min)	95.4 ± 0.3[a]	95.2 ± 0.7[a]	0.05 ± 0.00[a]	0.05 ± 0.01[a]
Soxhlet (6 h)	95.0 ± 0.4[a]	94.7 ± 0.2[a]	0.05 ± 0.00[a]	0.06 ± 0.00[a]

Mean ± SD. In each column, means with same letters are not significantly different ($p < 0.01$)

concentrations of linoleic and oleic acids in these oils were lower than those in oils extracted by the SFE system. On the other hand, punicic acid concentrations in the oils extracted by organic solvents (81.0–83.1%) were higher than those extracted by SFE system. Similar to punicic acid, the UFA level in the extracted oils by organic solvents was slightly higher than those extracted by the SFE System. The results from the FA compositions of the oils obtained by SFE indicated that these kinds of extractions can be more selective than those using the other methods applied in this study using organic solvents.

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