Pressurized Fluid Extraction of Pistachio Oil Using a Modified Supercritical Fluid Extractor and Factorial Design for Optimization

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Abstract

A pressurized fluid extraction (PFE) method for the extraction of pistachio oil was developed using a modified supercritical fluid extractor. The supercritical fluid extraction apparatus was modified to be able to pump liquid solvent and CO_2 into the extraction vessel alternatively. The experimental results showed that the extraction yield was independent of the extraction pressure. It was also noticed that addition of glass beads increased the extraction yield by more than 15%, while the extraction reproducibility expressed by RSD was improved from 4% to 1%. Furthermore, the use of glass beads reduced the solvent consumption from 35 to 20 mL. The influence of the effective variables (i.e. temperature, time, and glass beads percentage) was optimized by a factorial design method. The model allows the prediction of the extraction yield at different conditions. The PFE of pistachio oil was found comparable in terms of the extraction yield (52.6%) and fatty acid composition with the Soxhlet method. Two different solvents of n-hexane and ethanol were used for PFE of pistachio oil. The extraction yield was about one third (i.e. 18%) when ethanol was used as solvent.

Keywords: Pressurized Fluid Extraction; Accelerated Solvent Extraction; Pistachio Oil; Supercritical Fluid Extractor; Factorial Design Optimization; Soxhlet Extraction.

Introduction

Pistachio oil is a highly unsaturated emollient providing superior moisturization and a high level of nourishing essential fatty acids in a dry non-greasy, rapidly absorbed, and very low odor form. Pistachio nut contains more than 50% w/w oil from which 51-54% w/w is oleic acid and 31-35% w/w is linoleic acid (1).

Iran is one of the largest producers of pistachio. While a part of the harvested pistachios have no or only low marketable appearance and quality, they still have nutrition value. The oil of this kind of pistachio nuts has a good market and can be extracted and sold to increase the added value of pistachio cultivation.

Supercritical fluid extraction (SFE) has been previously used for extraction of oil from different nuts, such as canola (17), soybean (8), and peanut (10). SFE has also been used for the extraction of oil from hazelnut and pistachio at high pressures of 300-450 bar (15,16). In the case of pistachio oil extraction, even addition of 5-10% w/w of modifiers such as ethanol did not lead to lower operating pressure. Maximum recovery of pistachio oil was 66.14 % w/w of total oil at 345 bar and 60^{o}C in the presence of 10 w/w% ethanol (16).

Pressurized fluid extraction (PFE, Dionex trade name of Accelerated Solvent Extraction: ASE) is an efficient, rapid, selective, and reliable extraction method (4). PFE technology and its applications have been developed and used by various researchers (3,7,11,13). It has also been applied for

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the quantitative extraction of different samples of environmental organic compounds from soils (18) and lipids, and also for the analysis of food and biological samples (19). The possibility of changing several extraction variables such as temperature, pressure, and volume of solvent is a promising characteristic of newly available automated PFE instruments (14).

In this work, we have developed a new PFE method for the extraction of pistachio oil, using a modified SFE apparatus. A partial factorial design was used to design the experiment and a mathematical model was constructed to relate the extraction yield to the variables such as temperature, dynamic extraction time (i.e. proportional to the solvent consumption) and the percentage of added glass beads to the samples. The validity of the model was evaluated by analysis of variance (ANOVA) (12,16).

Materials and methods

Materials

Pistachio (Pistachia Vera L.) was obtained from Rafsanjan area of Iran. Ethanol, n-hexane, methanol, potassium hydroxide, hydrochloric acid and 14% w/w boron trifluoride in methanol were supplied by Merck Co.

Apparatus

The main components of the SFE apparatus has been described in detail elsewhere (9). For this work the apparatus was modified using a switching valve at the entrance of the pump to enable pumping of the liquid solvent and CO_2 alternatively into the extraction vessel. A gas chromatograph (CHROMPACK CP 9001, Netherlands) equipped with a capillary column (Sil 88, 50 m, 0.25 mm i.d.) and a flame ionization detector was used for the determination of fatty acid composition of extracted oil. An analytical balance (Sartorius, model LP 1200S, Germany) was used in gravimetric determination of the extraction yields on a moisture-free basis of the sample weight.

Sample preparation for PFE

The ground pistachio kernels passed through a sieve with mesh size of 16 and dried at 70^{o}C to a constant weight. It was kept within a sealed bag in a refrigerator until was used. The pistachio kernel samples were ground prior to PFE to facilitate analytes mass transfer during the extraction process. Since extraction kinetic is controlled by the kernel particle size; a sieving step is important to obtain reproducible extraction yield.

PFE procedure

The sample (~4 g) was loaded into the 10 mL stainless steel cell. Cotton wool was packed at the exit end of the cell to prevent transfer of solid samples to the tubing and clogging of the system. Two different solvents of n-hexane and ethanol were used to investigate the influence of solvent polarity on the yield of oil extraction from pistachio. The extraction program consisted static extraction, followed by dynamic extraction at a flow rate of 1 mL/min at different temperatures and pressures. The ground pistachio kernels were dispersed by different percentage of glass beads (Pyrex broken glassware were crushed and the portion was passed through mesh size of 40 was used). The solvent residue in the cell and tubing was removed with purging the PFE system with liquid CO_2 at the end of each extraction. The extraction solvent was evaporated at temperature of 50^{o}C. The extraction yield was calculated as the weight of extracted oil to the dry weight of the dried sample loaded into the extraction cell. In this work, the effect of different variables such as extraction temperature (40-80^{o}C), pressure (10-150 bar), dynamic extraction time (10-25 min) and the percentage of glass beads (20-60% w/w) were investigated. Preliminary experiments showed that increasing the pressure had only a negligible effect but the other variables had significant impacts on the extraction yields. Ethanol and n-hexane were used as solvent for PFE of pistachio oil. The extraction yield with n-hexane was found higher. Therefore, the extractions were performed with n-hexane at different temperatures, dynamic extraction times and the percentage of glass beads according to the factorial design procedure detailed below. Conventional soxhlet extraction (SE) was used as a reference method (15). Fatty acid methyl esters (FAME) of the extracted oils were prepared

and analyzed by GC-FID (2). Identification of FAME was based on retention times of reference compounds. Fatty acid composition was expressed as % of total FAME.

Factorial design

A three variables partial factorial design (temperature, T, dynamic time, t_d and the % glass beads mixed with ground pistachio nut, G) was used for the optimization of the extraction. From experiments that were carried out, total of 20 experiments were used in the design as listed in Table 1. The central point of the composition design was repeated 5 times. The three variables were tested at three levels, T: 50, 60, and 80^{o}C, t_d: 5, 10, and 15 min and G: 30, 50, and 60%. The program was written using MATLAB (the Math Works Inc., Version 6.0).

Table 1: The PFE experimental and calculated yields at different temperatures, % of glass beads, and dynamic extraction time by n-hexane at pressure of 10 bar, static extraction time of 5 min, and flow rate of 1 mL/min.

Temperature (^{o}C)	Glass beads (%)	Dynamic extraction time (min)	Experimental Yield (%)	Calculated Yield (%)
40	50	10	31.76	37.05
50	30	5	29.26	28.35
		10	34.47	34.88
		15	35.00	35.28
	60	5	34.60	36.00
		10	45.83	44.67
		15	47.87	47.19
60	50	5	42.00	41.05
		10	48.75	49.03
		10	50.00	49.03
		10	49.50	49.03
		10	49.40	49.03
		10	48.50	49.03
		10	48.60	49.03
		15	50.50	50.90
		20	49.00	46.63
		25	49.50	36.20
	20	10	33.90	38.94
	40	10	48.10	46.71
	60	10	49.40	50.32
80	30	5	41.57	42.23
		10	48.93	48.89
		15	50.43	49.43
	60	5	42.34	41.45
		10	50.26	50.23
		15	52.58	52.88

Results and discussion

The effect of extraction temperature

Increasing temperature of the extraction increased the extraction yield as shown in Fig. 1. An extraction temperature of 80^{o}C was good enough to give 100% oil recovery. The increase in yield with temperature is due to increase in solvating power at higher temperatures. Moreover, the viscosity of organic solvents decreases at higher temperatures and the solvent penetrate better into the matrix and hence an improvement in the extraction yield is observed (5)**.** The extraction yield can also be increased by increasing the solvent throughput (i.e. proportional to t_d at constant flow rate). This

method was preferred instead of working at the high temperatures.

The effect of static and dynamic extraction time

A 5 min period of static extraction time improved the overall yield of the oil extraction. While at the higher static extraction time of 10 minutes no improvement on the extraction yield were observed. Therefore, the static time of 5 min was used for all the experiments. Profound effect on the extraction yield was observed when the extraction solvent was passed through the sample cell for a period of time (i.e. called dynamic extraction time). Since a constant flow rate of solvent is used, by increasing the extraction time the amount of solvent passing through the extractor is also increased. As shown in Fig. 2, with a 15 mL of solvent, the extraction yield is increased to 48.6%. This results show that the extraction time in PFE method is about 20% of the SE and SFE methods (16).

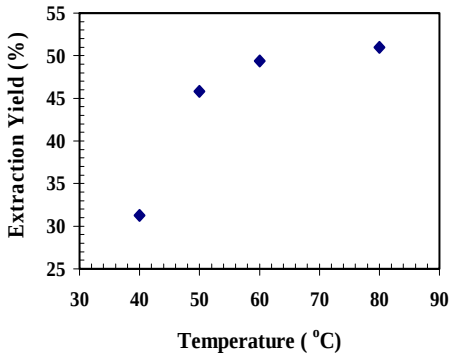

Fig. 1. The extraction yield versus temperature at pressure of 10 bar, glass beads of 50%, ground pistachio kernel of 4 g, static extraction time of 5 min, dynamic extraction time of 10 min, flow rate of 1 mL/min, and n-hexane as solvent.

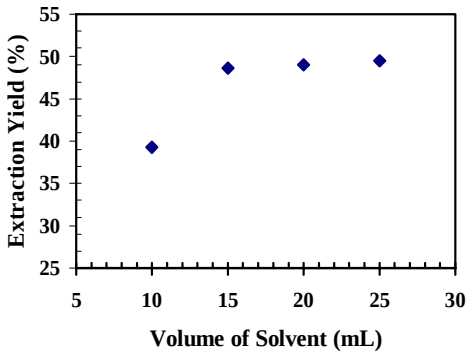

Fig. 2.The extraction yield versus volume of n-hexane as solvent at temperature of 60°C, pressure of 10 bar, glass beads of 50%, ground pistachio kernel of 4 g, static extraction time of 5 min, and flow rate of 1 mL/min.

The effect of addition of glass beads to the sample

The extraction yield as a function of the percentage of glass beads mixed with the sample (w/w) is shown in Fig. 3. The method of packing of the sample in the cell and the contact surface between the sample and the solvent is very important (5). Therefore, the pistachio kernels were ground and well mixed with the glass beads. Addition of the glass beads increased the extraction yield by more than 15%, and decreased RSD of the extraction from 4 to 1%. Furthermore, it considerably decreased the solvent consumption. The glass beads mixed with the ground pistachio disperses the matrix and thus increases the contact surface of the sample and the solvent, and it improves the homogeneity of the sample packed into the extraction solvent.

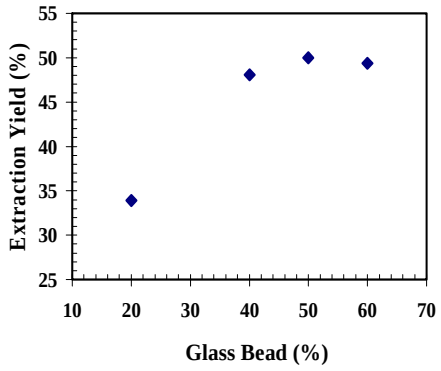

Fig. 3. The extraction yield versus the % of glass beads mixed with the sample at temperature of 60°C, pressure of 10 bar, ground pistachio kernel of 4 g, static extraction time of 5 min, dynamic extraction time of 10 min, flow rate of 1 mL/min, and n-hexane as solvent.

The effect of extraction solvent

Two different solvents were used for the extraction. At similar conditions, the yield of extraction by ethanol was found to be about 66% lower than the yield of extraction with n-hexane (Table 2). Triglycerides and n-hexane are fully miscible while ethanol and triglycerides are not and this leads to higher extraction yields for n-hexane in comparison with ethanol. The other advantage of n-hexane in comparison with ethanol is its lower boiling point that leads to easier evaporation of n-hexane at the end of each extraction. The separation of solvent residue, at the end of extraction could be performed by liquid

CO_2. Both n-hexane and ethanol has a high solubility in liquid or supercritical CO_2 while triglycerides have relatively limited solubility, therefore the solvent residue could also be extracted from the pistachio oil as reported by Eller, et al(6) for the extraction of n-hexane residue from soybean oil. Depending on the solvent used the color of oil was different. With using ethanol as extraction solvent, the color of oil was green, that is probably due to the co-extraction of chlorophyll, while the color of oil was yellow when n-hexane was used as solvent.

Table 2: The PFE yield (%) of pistachio oil with n-hexane and ethanol as solvent at pressure of 10 bar, temperature of 50°C, static time of 5 min, glass beads of 30%, flow rate of 1 mL/min, at different dynamic times.

Type of solvent	Dynamic time (min)		
	5	10	15
Ethanol	9.6	12.0	15.0
n-Hexane	29.3	34.5	35.0

The effect of pressure

The PFE was performed at the pressure range of (10-150) bar, but a small change was observed on the extraction yield. Therefore, pressure of 10 bar was applied only to keep the solvent as liquid at the high temperatures and drive the solvent through the sample in order to improve the extraction yield.

Modeling

A second–order polynomial equation is proposed for prediction of the extraction yields from pistachio oil by n-hexane as a function of independent variables as follows:

$$Y = a_o + a_1.T + a_2.t_d + a_3.G + a_4T.t_d + a_5.T.G + a_6.t_d.G + a_7.T^2 + a_8.t_d^2 + a_9.G^2 \quad (1)$$

where a_0 is the intercept; a_1, a_2, . . . , a_9 are the coefficients of the polynomial; Y is the extraction yield (% w/w); T is the temperature; t_d is the dynamic extraction time, and G is the % of glass beads mixed with the sample. The coefficients of these variables were obtained by multiple least-squares regression, using the MATLAB program. For each coefficient the t value for the null hypothesis (H_o) were calculated. If t value for each coefficient was greater than the critical value, the variable had significant effect in the model (at confidence limit of 95%), and was preserved. After performing t-test the following equation was obtained:

$$Y = -112.2294 + 3.2101T + 2.6751\ t_d + 1.1235\ G + 0.0009\ T\ .t_d - 0.0094\ T.G + 0.0141\ t_d\ .G - 0.019\ T^2 - 0.1225\ t_d^2 - 0.0052\ G^2 \quad (2)$$

As shown in Equation 2, all variables proposed in Equation 1, have significant effects in the model. Temperature, dynamic extraction time, and % of glass beads have a significant effect on the response, but the second-order interactions are less important. Response surfaces showing the effects of different variables on the extraction yields are given in Figs. 4-6. An ANOVA was used to evaluate the validity of the model. There was no lack of fit at the confidence limit of 95% (calculated F lack of fit is less than the critical value, 3.39 < 4.95). The correlation coefficient of the extraction yield calculated from the model with the experimental extraction yield is $R^2 = 0.9896$ for the data shown in Table 1, which indicates good performance of the model. The maximum extraction yield, under different conditions was predicted, using the program written by MATLAB.

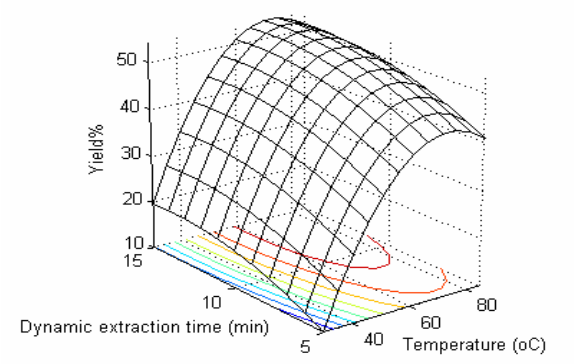

Fig.4. Response surface of the extraction yield (%) versus temperature and dynamic extraction time at the 50% glass beads.

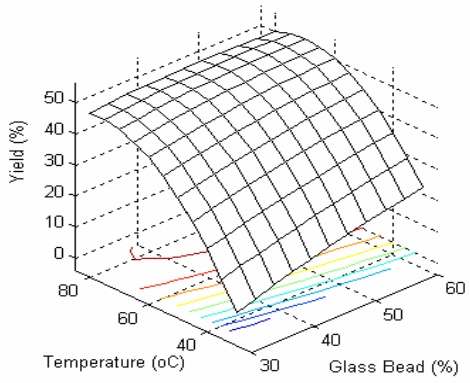

Fig. 5. Response surface of the extraction yield (%) versus temperature and % glass beads at the dynamic extraction time of 10 min.

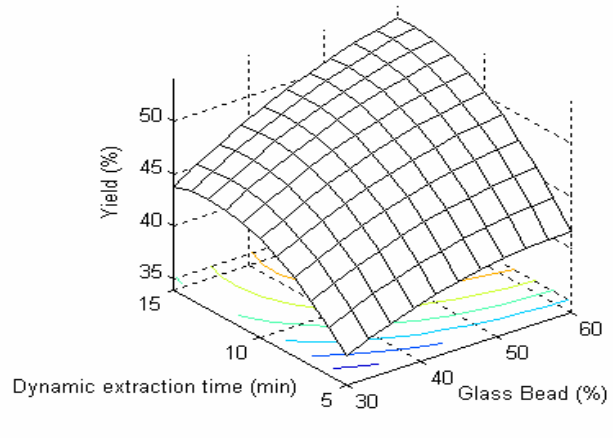

Fig. 6. Response surface of the extraction yield (%) versus dynamic extraction time and % glass bead at the temperature of 60°C.

Comparison of SE, SFE, and PFE methods

Methods of SE, SFE and PFE have been compared in terms of type of solvent, solvent consumption per g of sample, temperature, process time, and the extraction yield in Table 3. PFE is superior in terms of solvent consumption and process time while having a high extraction yield.

The results shown in Table 4 indicate that the oil fatty acid composition expressed as % of total fatty acid methyl esters, extracted by SE and PFE methods are similar.

Table 3: Comparison of Pressurized Fluid Extraction (PFE) method with Soxhlet and Supercritical Fluid Extraction (SFE) methods for the extraction of pistachio oil.

Parameters	Soxhlet[1]	SFE[2]	PFE
Type of Solvent	n-Hexane	CO_2	n-Hexane
Solvent/Sample Weight (mL/g)	10	2000[3]	5
Temperature (°C)	68	60	60
Process Time (h)	2.5	2.5	~ 0.5
Extraction Yield (%)	52.6	34	52.6

1 This work

2 Palazoglu & Balaban, 1998

3 Expanded CO_2 gas volume (mL) per g sample

Table 4 : The main fatty acid composition of oil (% w/w of identified fatty acids) extracted by PFE and SE methods.

Fatty acid	%	
	PFE	SE
C16:0	9.46	9.17
C18:0	1.36	1.23
C18:1	54.73	55.11
C18:2	29.06	29.45
C18:3	0.37	0.37

Conclusions

The oil from low market value pistachio nuts was extracted by PFE method with the overall yield of 52.6% that is equal to 100% oil recovery with lower solvent consumption and the extraction time in comparison with the SE method. The fatty acid composition of pistachio oil extracted by PFE method was found similar to that of the oil extracted with SE method.

The proposed model can predict the maximum extraction yield at different conditions. Mixing of glass beads with the sample increased the extraction yield by more than 15%, and decreased RSD of the extraction from 4 to 1% and lowered the extraction solvent consumption from about 35 to 20 mL. Extraction yield was about one third, when ethanol was used as the extraction solvent.

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استخراج سیال تحت فشار روغن پسته با استفاده از یک استخراج کننده سیال فوق بحرانی اصلاح شده وطرح آماری برای بهینه سازی

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چکیده

در این مقاله استخراج روغن پسته با روش سیال تحت فشار، در دستگاه استخراج با سیال فوق بحرانی معرفی شده است. با اصلاح دستگاه مذکور حلال مایع و گاز دی اکسید کربن بطور متناوب به داخل سیستم پمپ می شوند. نتایج آزمایشات نشان می دهد که راندمان استخراج روغن پسته مستقل از فشار می باشد. راندمان استخراج با اضافه کردن خرده شیشه به نمونه، بیشتر از ۱۵٪ افزایش یافته و همچنین تکرارپذیری (انحراف استاندارد نسبی) از ۴٪ به ۱٪ بهبود می یابد. علاوه بر این میزان مصرف حلال برای استخراج روغن در اثر استفاده از خرده شیشه کاهش می یابد. اثر متغیرهای (دما، زمان و در صد خرده شیشه) با استفاده از روش طراحی فاکتوریال اپتیمم و مدلی برای پیش گوئی راندمان استخراج در شرایط متفاوت ارائه گردید. روغن پسته حاصل از روش استخراج با سیال تحت فشار از لحاظ راندمان استخراج (۵۲/۶٪) و ترکیب اسیدهای چرب با روش سوکسله قابل مقایسه می باشد. برای استخراج روغن پسته با روش سیال تحت فشار، اتانول و هگزان نرمال بعنوان حلال مورد استفاده قرار گرفتند که راندمان استخراج با اتانول حدود یک سوم (۱۸٪) راندمان با هگزان نرمال گردید.

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