



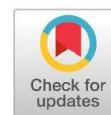
Chemical Methodologies

Journal homepage: <http://chemmethod.com>



Original Research article

Extraction and Determination of Essential Oil of *Arachis hypogaea* through Supercritical Fluid Extraction Coupled with GC-MS



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ARTICLE INFORMATION

Received: 26 May 2020

Received in revised: 04 July 2020

Accepted: 10 August 2020

DOI: [10.22034/chemm.2020.113872](https://doi.org/10.22034/chemm.2020.113872)

KEYWORDS

Peanut

Supercritical fluid extraction

GC-MS

Essential oil

Optimization

ABSTRACT

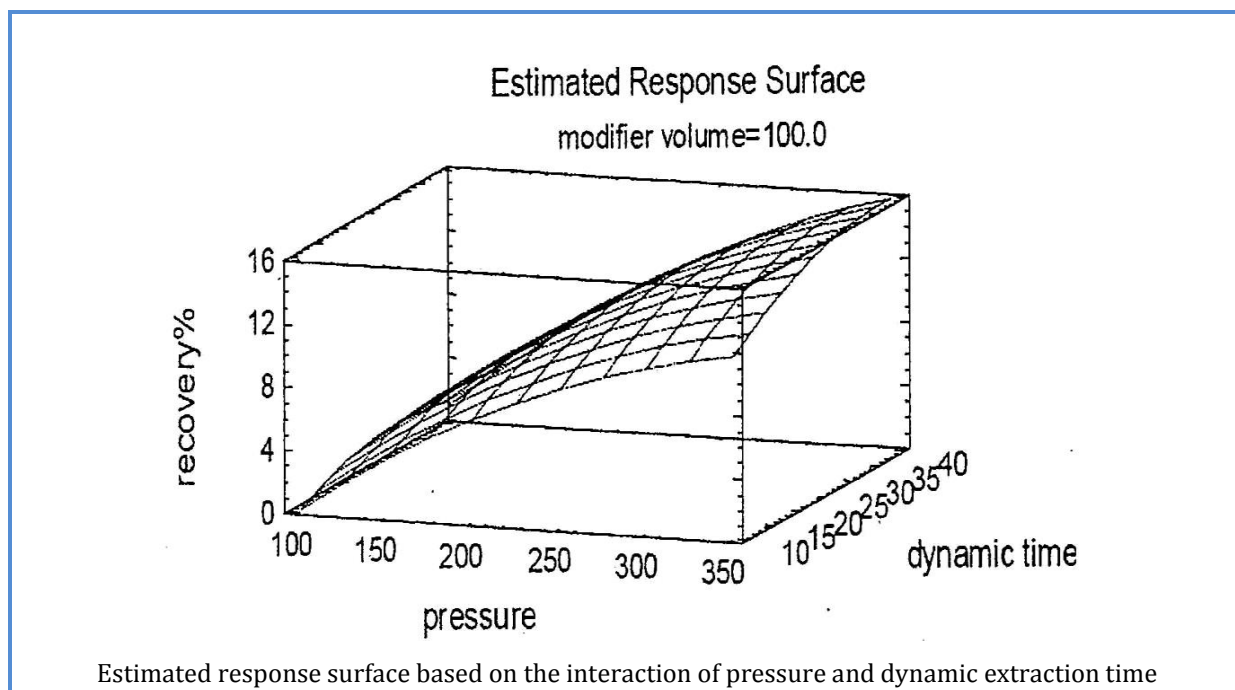
Peanut essential oils cover a big part of the human diet, containing various proteins and lipids. Optimized extraction of essential oils from peanut could acts an important role in food and pharmaceutical industries. The aim of this study was to validate a cost-effective green method with high recovery rates for extracting the peanut essential oils. Supercritical fluid extraction (SFE) was implemented for the extraction of oils from *Arachis hypogaea*. The experimental parameters of SFE such as pressure, temperature, modifier volume, static, and dynamic extraction time were optimized using a central composite design after a 2n-1 fractional factorial design. Chemical compositions of the SFE extract were characterized using the GC-MS. Optimum conditions for supercritical fluid extraction were the temperature of 65 °C, 10 min time for static extraction, the pressure of 350 atm, dynamic extraction time of 35 min, and modifier volume of 150 µL. Major components of the SFE extracted oils in optimum conditions were oleic acid (35%), linolic acid (7.4%), palmitic acid (5%), and stearic acid (4.5%). The extraction recovery based on the SFE varied at the range of 0.60–17.10% (w/w) under different conditions. SFE coupled with GC-MS was successfully implemented for extraction of the peanut essential oils and it is a green and cost-effective method which could potentially replace the existing methods in the food and pharmaceutical industry.

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Graphical Abstract



Introduction

Essential oils are so critical for health that any resource could be fundamental. Peanut essential oils comprise a huge part of the human diet and it contains various proteins and lipid [1-3]. Approximately, 29% (w/w) and 50% (w/w) of peanut are protein and oil, respectively. Conventionally, oil is extracted from peanuts either by mechanical pressing or solvent extraction [4-7]. Due to the low oil recovery of the mechanical pressing method it is considered less efficient. Therefore, more efficient and cost-effective methods for the extraction of the peanut essential oil are in demand. Also, extraction must be easily optimized to maximize the amount of essential oil that has been extracted from natural sources such as peanuts [8, 9].

Supercritical fluid extraction (SFE) has recently become popular for the extraction of essential oils from the aromatic plants [1, 2, 10, 11]. This method offers effective and rapid extraction, necessitates just moderate temperatures, with no clean-up steps and the use of harmful organic solvents. 6 carbon dioxide (CO_2) is a great solvent for extraction and isolation of essential oils from plants, which is nonexplosive, available, non-toxic and easily removed from the extracted products [12-16]. Generally, the efficiency of SFE depends on pressure, temperature, dynamic and static extraction time and modifier volume [17-21].

Central composite designs, abbreviated as CCD, or Box-Wilson, is one of the most common methods of response level. The CCD method is similar to the factor or factor design of a fraction that includes centre points. The star points are used to estimate curvature. The number of star points is twice the number of factors ($2k$). If the distance from the centre point of the design space to the operating points (square corners) is the same for each factor, the distance from the centre point to the star points (α) will be greater than one. Therefore, CCD design is usually done on five levels, including α , -1 , 0 , $+1$, $-\alpha$, -1 , 0 and $+1$ are the upper and lower levels and $-\alpha$ and $+\alpha$ are the new limiting factors. Zero is also the centerpiece of design. The exact amount of alpha (α) depends on the design feature and the number of factors. Figure 2 shows the geometry of the two-factor central compound design with a combination of factor design with central points and star points (22). To maintain the rotatability, the value of α depends on the number of experiments in the general design section of the CCD and is calculated as equation 1.

$$\alpha = [\text{Number of factor design experiments}]^{1/4} = [2k]^{1/4} \quad (2)$$

Therefore, for a complete factor design, the second equation is true, where k is the number of factors for the complete factor design. Based on the location of the star points, three different methods of CCD are defined, which are:

Circumscribed central composite: Limited central compound design, or CCC, is the main form of central compound design in which the star points and therefore the alpha value depends on the properties and the number of factors. In this type of useful design, the alpha is larger than one. Star points determine a new range of higher and lower levels for factors. Therefore, the factors are considered as five levels. This design has hyper spherical symmetry [23].

Inscribed central composite: In cases where there is a special limitation for factor levels, a centralized composite design or CCI is used. Due to this limitation, star points are considered as factors in the factor design section. In other words, the CCI method is a reduced scale of the CCC method in which the values of $1/\alpha$ instead of α are used. Therefore, this design is also performed on five levels including, $+\alpha$, 0 , -1 , 0 , $+1$, and $-\alpha$ [24].

Face-centered central composite: The CCF method, which is a special design of the CCD set of star dots, is at the centre of every aspect of factor design (Figure 3). The alpha value in this design is equal to one, so it is a three-level design [25, 26].

In this research study, peanut seeds were exposed to the supercritical CO_2 using the SFE method. Then, essential oils were collected and injected into GC-mass for characterization and quantitation. Every step was optimized using a central composite design and factorial design [23-25].

Experimental

Materials and methods

99.89% purity of carbon dioxide was purchased from the Sabalan Co (Tehran, Iran). Methanol with a high degree of purity and 99.8-99.00% ethanol were purchased from Merck and Bidestan, respectively. The peanuts were sampled from Gilan province (Iran) and ground to a diameter of about 0.5 mm using a domestic mill.

Extraction was performed using the supercritical fluid by SFE machine of MPS/225 from Suprex and Pittsburgh, equipped with a syringe pump from Suprex and an extraction cell made of stainless steel with a volume of 0.3 mL. The compressor from Suprex company was used to collect the extracted sample to warm up to 75 °C to prevent the freezing of the path.

Identification of the extracted materials was performed using a gas chromatography machine made by HP integrated with the HP trace type spectroscopy equipped with a four-pole analyzer and a Hp-5 column (30 mm×25 mm i.d.). Mass spectra were obtained using an electron-ionizing ionization source with energy of 70 electrons.

SFE

Extraction using supercritical fluid was performed using the SFE machine model MPS/225 from Suprex and Pittsburgh equipped with a syringe pump from Suprex and extraction tubes of stainless steel with a volume of 0.3 mL. The compressor from Suprex company was used to collect the extracted sample to warm up to 75 °C to prevent the freezing of the path.

GC/MS

In this study, GC/MS was used to detect the volatile compounds in the essential oil extracted from peanut seed by supercritical fluid CO₂. Identification of the extracted materials was performed by a gas chromatography machine made by HP integrated with the HP trace type Spectroscopy equipped with a four-pole analyzer and an Hp-5 column (30 mm×25 mm i.d.). Mass spectra were obtained using an electron-ionizing ionization source with energy of 70 electrons.

Method

Sample preparation

The sample preparation was done through washing the received peanut with double distilled water. The washed samples were dried through purging Helium gas for 5 min.

Supercritical fluid extraction (SFE)

Exactly 5000 mg peanut was mixed with glass beads and extracted into tubes. As a result, the contact surface of the compound with the supercritical fluid is increased and the tunneling phenomenon is prevented. 150 μ L methanol was added directly to the cell. After adjusting the pressure at 350 atm at 35 $^{\circ}$ C, the static extraction time at 10 min and dynamic extraction time at 35 min, the inlet fluid of the supercritical fluid was opened and the system was extracted to the desired condition. During the static period, the sample is in contact with the fluid for a certain time at the desired temperature and pressure. In a dynamic state, the fluid continuously passes through the sample at the specific rate, so that the dissolved compounds can get carried into the collecting cell. The flow velocity was 0.3 ± 0.05 mL/min and the extracted materials were mixed in 1.0 mL of ethanol in a 0.2 mL volume flask. In the end, the final volume was extracted into 0.2 mL ethanol. In order to improve the collection efficiency, the container was placed in the ice bath during the time of dynamic extraction. After evaporation of ethanol at room temperature, the extraction efficiency was obtained by weighing the residual materials [27-31].

Optimization methods

Fractional factorial design (FFD)

Five variables that could have an impact on the extraction efficiency: pressure (A), dynamic extraction time (B), modifier volume (C), temperature (D) and static extraction time (E) were used to design 18 experiments using the FFD method. The low and high levels of the parameters were used to screen these parameters and find the optimum level. A Pareto chart was plotted to evaluate the result of the interaction between parameters and the significance of each of them on the final extraction recovery (Table 1).

Table 1. Variables at a low and high level for screening test

| Variable | Low | High |
|-----------------------------|-----|------|
| Pressure (atm) | 100 | 350 |
| Temperature ($^{\circ}$ C) | 35 | 65 |
| Modifier volume (μ L) | 0 | 100 |
| Dynamic time (min) | 10 | 40 |
| Static time (min) | 10 | 40 |

Central composite design (CCD)

After the screening process, the most significant variables were chosen for CCD. 16 experiments were conducted accordingly. Based on the results, the systemic error was checked and the random

distribution of the data was analyzed. The optimum level of each variable was determined based on the results. All these analyses were done using the statgraphics 5.1 software. As seen in Figure 1, the pressure, dynamic time and modifier volume were the independent variables with the highest effect on the extraction.

GC-Mass detection and determination

Gas chromatography with mass spectrometry was used to determine and characterize the essential oils extracted from the peanuts. After collecting the essential oil and adding the required amount of ethanol, 1.0 μL solution was injected to GC-MS. The identification of the compounds was done by comparing the retention time to the ones reported in the literature. Also, the mass spectra were obtained and compared with the standard data and valid literature. The temperature was set at 60 $^{\circ}\text{C}$ for 3 min and increased up to 280 $^{\circ}\text{C}$ with a rate of 5 $^{\circ}\text{C}/\text{min}$. The temperature of the injection site was set at 270 $^{\circ}\text{C}$ [20, 31-37].

Results and discussion

FFD and CCD results

Using a fractional factorial design 18 experiments were carried out and a Pareto chart was prepared to assess the effect of these variables on extraction efficiency with a confidence interval of 95% (Table 2). The interaction of parameters and their simultaneous effect on the extraction efficiency were examined 2 by 2, which this calculation is illustrated in the Pareto chart (Figure 1).

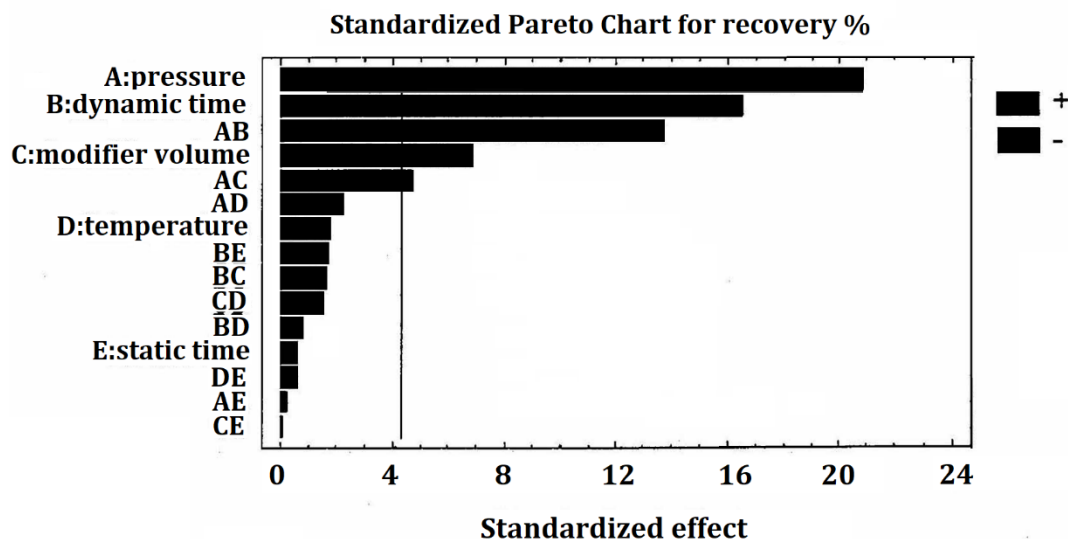


Figure 1. Pareto graphs obtained from the screening stage. A: Pressure, B: Dynamic time, AB: Interaction between pressure and dynamic time, C: Modifier volume

Table 2. Screening test results designed using the fractional factorial method

| Extraction recovery (W/W) | Static time (min) | Temperature (°C) | Modifier volume (uL) | Dynamic time (min) | Pressure (atm) | No. |
|---------------------------|-------------------|------------------|----------------------|--------------------|----------------|-----|
| 5.20 | 25 | 50 | 50 | 25 | 225 | 1 |
| 0.98 | 40 | 65 | 100 | 10 | 100 | 2 |
| 9.31 | 10 | 65 | 100 | 10 | 350 | 3 |
| 0.14 | 10 | 65 | 100 | 10 | 100 | 4 |
| 1.10 | 40 | 35 | 0 | 10 | 100 | 5 |
| 5.30 | 25 | 50 | 50 | 25 | 225 | 6 |
| 0.46 | 40 | 65 | 0 | 10 | 350 | 7 |
| 10.82 | 10 | 65 | 0 | 40 | 350 | 8 |
| 1.66 | 40 | 35 | 0 | 40 | 100 | 9 |
| 16.78 | 40 | 65 | 100 | 40 | 350 | 10 |
| 0.28 | 40 | 65 | 100 | 40 | 100 | 11 |
| 10.80 | 40 | 35 | 0 | 40 | 350 | 12 |
| 1.21 | 10 | 65 | 100 | 40 | 100 | 13 |
| 0.99 | 10 | 35 | 100 | 10 | 100 | 14 |
| 9.32 | 40 | 35 | 100 | 10 | 350 | 15 |
| 16.10 | 10 | 35 | 100 | 40 | 350 | 16 |
| 1.20 | 10 | 35 | 100 | 40 | 100 | 17 |
| 0.44 | 10 | 35 | 0 | 10 | 100 | 18 |

According to the results obtained at the screening stage, two parameters of temperature and time of static extraction were kept constant at 65 °C and 10 min, respectively, to continue the optimization (Figure 2).

Based on the results obtained by fractional factorial design, three parameters (pressure, modifier volume, and dynamic extraction time) were identified as significant for the SFE optimization. Based on the CCD designed using these 3 parameters, 16 experiments were implemented using low, centre and high levels of these variables (Table 3). The results are presented in Table 4. Based on the residual plot for recovery after CCD, due to the random distribution of the results, it can be concluded that there was no systemic error (Figure 2).

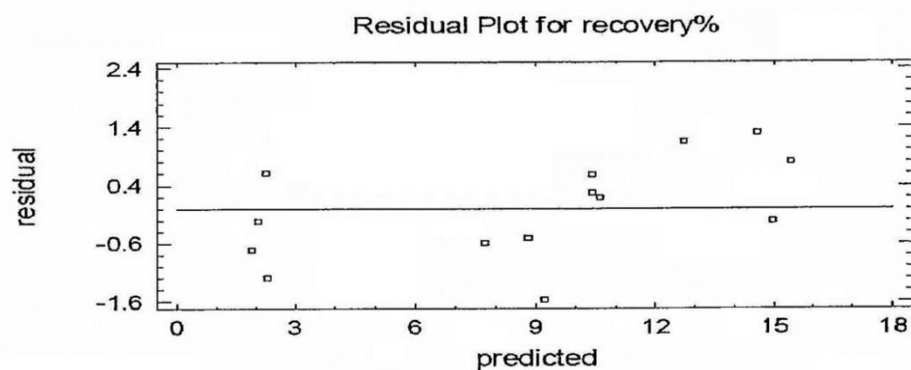
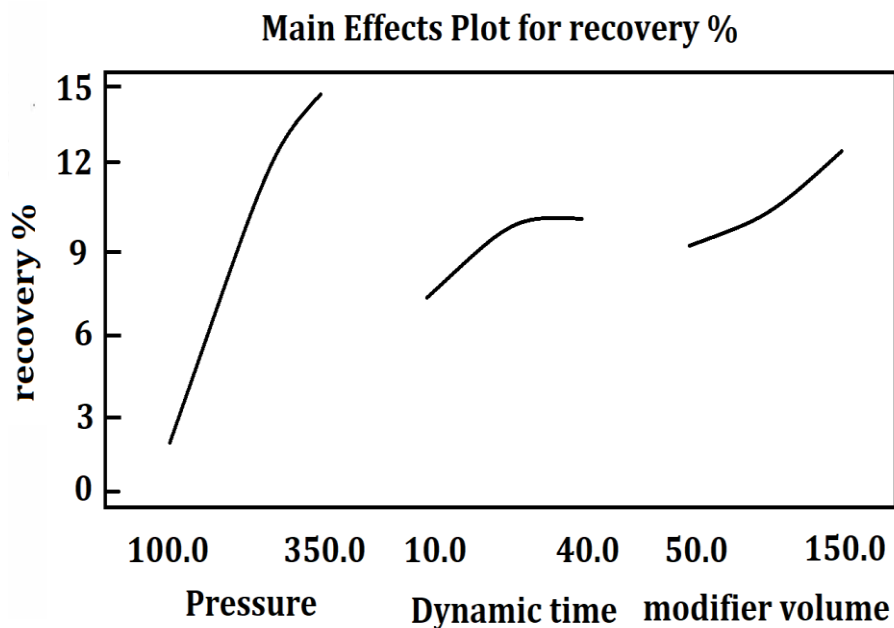
**Figure 2.** Residual plot for recovery based on CCD results

Table 3. Low, centre, and high level of the selected parameters for optimization using CCD

| Variable | Low | Centre | High |
|-----------------------------------|-----|--------|------|
| Pressure (atm) | 100 | 225 | 350 |
| modifier volume (μL) | 50 | 100 | 150 |
| dynamic time (min) | 10 | 25 | 40 |

Optimum condition for SFE

Pressure, time of dynamic extraction and modifier volume had a significant effect on the recovery rate (p -value <0.05). By increasing pressure from 100 to 350 atm the recovery rate was increased. Therefore 350 atm was chosen as the optimum level (Table 5 and Figure 3). As the duration of dynamic extraction increased, the recovery rate was shown to plummet after 35 min. As a result, 35 min was selected as the optimum time as the equilibrium was being disturbed and the essential oil was being transported along with the other components.

**Figure 3.** The effect of pressure, dynamic time, and modifier volume on SFE after CCD

The estimated response surface graph for two parameters of pressure and dynamic time is demonstrated in Figure 4, while the modifier volume is constant at 100 μL . As seen in Figure 4, by increasing the time from 10 min to 35 min, the extraction efficiency was increased, and by increasing the time from 35 to 40 min the extraction efficiency was decreased. During the extraction of the dynamic time, the supercritical fluid passed through the sample continuously.

Table 4. CCD and the results obtained for the significant parameters affecting SFE efficiency

| No. | Pressure (atm) | Dynamic time (min) | Modifier volume (μL) | Recovery (W/W) |
|-----|----------------|--------------------|-----------------------------------|----------------|
| 1 | 225 | 25 | 100 | 10.98 |
| 2 | 350 | 40 | 150 | 17.10 |
| 3 | 225 | 40 | 100 | 10.78 |
| 4 | 225 | 25 | 150 | 13.87 |
| 5 | 350 | 10 | 150 | 16.23 |
| 6 | 100 | 25 | 100 | 1.86 |
| 7 | 350 | 10 | 50 | 8.31 |
| 8 | 100 | 10 | 150 | 1.11 |
| 9 | 350 | 25 | 100 | 14.78 |
| 10 | 100 | 10 | 50 | 0.68 |
| 11 | 100 | 40 | 150 | 2.7 |
| 12 | 225 | 10 | 100 | 7.12 |
| 13 | 100 | 40 | 50 | 1.18 |
| 14 | 225 | 25 | 50 | 7.67 |
| 15 | 350 | 40 | 50 | 15.90 |
| 16 | 225 | 25 | 100 | 10.76 |

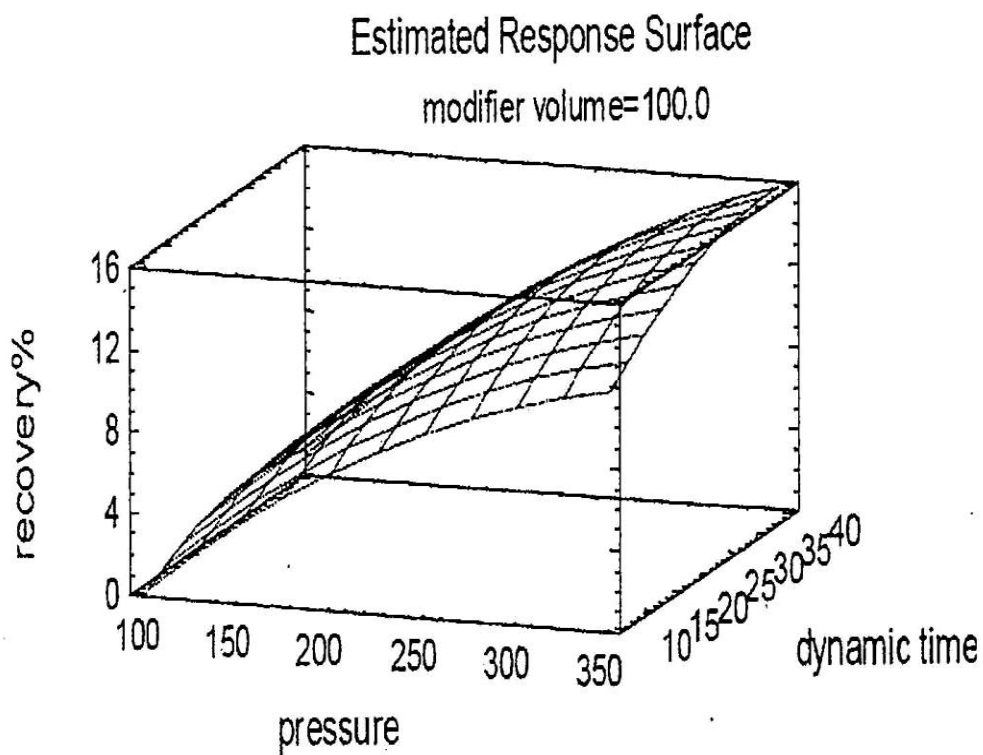
**Figure 4.** Estimated response surface based on the interaction of pressure and dynamic extraction time

Table 5. The significant parameter on the efficiency of SFE

| Source | Sum of square | Df | Mean square | F-ratio | P-value* |
|--------------------|---------------|----|-------------|---------|----------|
| A: pressure | 417.574 | 1 | 417.574 | 72.18 | 0.0000 |
| B: dynamic time | 20.678 | 1 | 20.678 | 9.10 | 0.0235 |
| C: modifier volume | 30.415 | 1 | 30.415 | 13.38 | 0.0106 |
| AA | 9.227 | 1 | 9.227 | 4.06 | 0.0905 |
| AB | 4.805 | 1 | 4.805 | 2.11 | 0.1962 |
| AC | 6.125 | 1 | 6.125 | 2.69 | 0.1518 |
| BB | 4.0593 | 1 | 4.059 | 1.79 | 0.2299 |
| BC | 3.726 | 1 | 3.726 | 1.64 | 0.2477 |
| CC | 0.884 | 1 | 0.884 | 0.39 | 0.5558 |
| Total error | 13.637 | 6 | 2.272 | | |

*P-value <0.05 is significant

Modifier volume is also of great importance as it can be absorbed into the sample, increasing the recovery rate of the essential oil. By increasing the modifier volume from 50 to 150 μL , the recovery rate was raised. So, a 150 μL modifier volume was chosen for the final sample analysis.

In Figure 5, the estimated response surface reflects the two parameters of pressure and modifier volume during the dynamic time of 25 min. Methanol is the most commonly used modifier in SFE. There is a major modification to the use of the modifier: the solvent modifier changes the analyte molecules in the supercritical fluid by changing the polarity of it, b) the modifier can interact with the sample and increase penetration in the sample. This action increased the active sites for the dissolution of the analyte into the fluid, causing a significant increase at the extraction efficiency. The contour in Figure 6 shows the optimum level marked as plus at a constant modifier volume level of 100 μL . However, the duration of static extraction and temperature had no significant effect on the optimization of the process, which is why 10 min and 65 $^{\circ}\text{C}$ were chosen for the rest of the experiments, respectively.

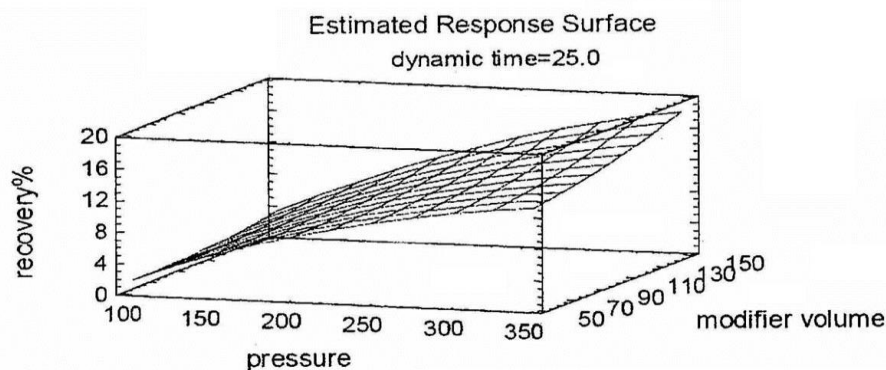


Figure 5. Estimated response surface based on the interaction of pressure and modifier value during a dynamic extraction time of 25 min

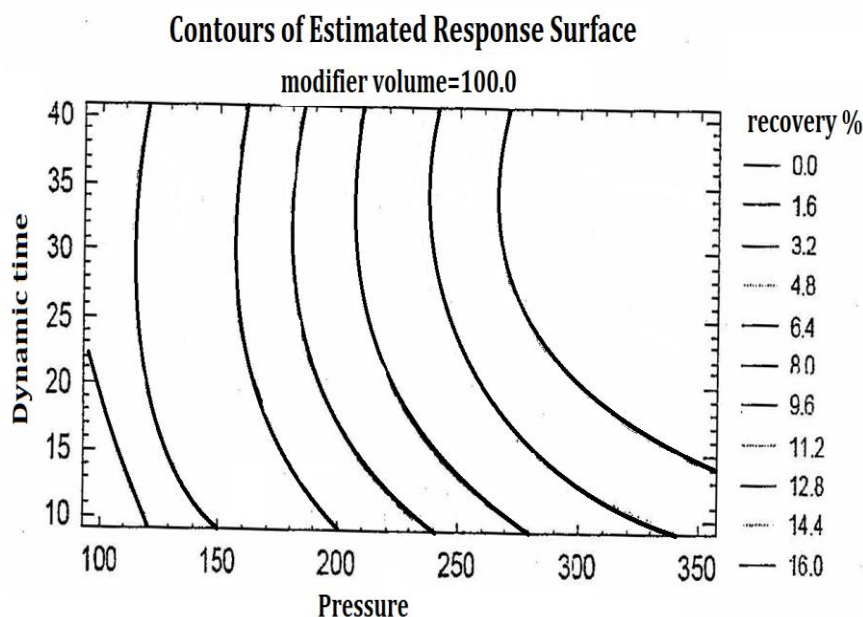


Figure 6. Contours of estimated response surface exhibiting the interaction of the dominant parameters on the extraction efficiency: dynamic extraction time and pressure (modifier volume=100 μ L), + shows the optimum level

Results of GC/MS analysis

Under optimum conditions (as seen in Table 6), 14 compounds containing more than 95% of the compounds extracted from peanut seeds were identified. The extracted essential oil mainly contains oleic acid (35%), linoleic acid (7.4%), stearic acid (4.5%), palmitic acid (5%), and 2,4-Decadienal (3%).

It is noteworthy that this cost-effective technique is more efficient when it comes to extracting and isolating essential oils in the case of peanut. As peanut is one of the most popular sources of essential oil, SFE could bear the potential to replace conventional methods. It is more practical and less labor-intensive. It requires lower temperature compared to the other methods. Nevertheless, it can be optimized as can be seen in this study and discussed by Yousefi *et al.* [11] in a review article pinpointing the overall advantages of SFE and its superiority to another method. Accordingly, the essential oils extracted from the peanut, which is abundant and cheaper compared to other resources, could be beneficial in the pharmaceutical industry. However, the health benefits of these oils cannot be overlooked at any cost. Hopefully, the results obtained in this study would be useful for food and pharmaceutical industries to lower the cost and increase the efficiency.

Table 6. Characterized components of peanut after GC-mass analysis

| No. | Compound | Area |
|-----|-------------------|------|
| 1 | Hexanal | 5% |
| 2 | α - pinene | 7% |
| 3 | Pentyl furan | 6.5% |
| 4 | 2- octanone | 3% |
| 5 | Octanal | 8% |
| 6 | 2,4- hepadienal | 11% |
| 7 | Nonenal | 1.1% |
| 8 | 2-nonenal | 1% |
| 9 | Menthol | 2% |
| 10 | 2,4- decadienal | 3% |
| 11 | Stearic acid | 4.5% |
| 12 | Oleic acid | 35% |
| 13 | Linolic acid | 7.4% |
| 14 | Palmetic acid | 5% |

Table 7. The recovery rate of essential oils extracted from peanut using SFE detected by GC-mass

| Runs No. | Pressure | Dynamic time | Modifier volume | Recovery (%) |
|----------|----------|--------------|-----------------|--------------|
| 1 | 225 | 25 | 100 | 10.98 |
| 2 | 350 | 40 | 150 | 17.10 |
| 3 | 225 | 40 | 100 | 10.78 |
| 4 | 225 | 25 | 150 | 13.87 |
| 5 | 350 | 10 | 150 | 16.23 |
| 6 | 100 | 25 | 100 | 1.86 |
| 7 | 350 | 10 | 50 | 8.31 |
| 8 | 100 | 10 | 150 | 1.11 |
| 9 | 350 | 25 | 100 | 17.1 |
| 10 | 100 | 10 | 50 | 0.68 |
| 11 | 100 | 40 | 150 | 2.87 |
| 12 | 225 | 10 | 100 | 7.12 |
| 13 | 100 | 40 | 50 | 1.18 |
| 14 | 225 | 25 | 50 | 7.67 |
| 15 | 350 | 40 | 50 | 15.90 |
| 16 | 225 | 25 | 100 | 10.67 |

Conclusion

Essential oils in peanut seed were successfully extracted and isolated using the SFE method. They were also characterized and determined by GC-MS. The majority of the detected oils were oleic acid (35%), linoleic acid (7.4%), stearic acid (4.5%), palmitic acid (5%), and 2,4- Decadienal (3%). Based on the optimum results, this method offers desirable recovery rates that can be used in food and pharmaceutical industries for the maximum extraction of essential oils.

Acknowledgements

The authors appreciate the Islamic Azad University Pharmaceutical Sciences Branch, Tehran, Iran for their great support.

Conflict of Interest

The Author(s) declare(s) that there is no conflict of interest.

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How to cite this manuscript: Nastaran Rahimi, Alireza Narenjian, Mahnaz Qomi*, Mohammad Hossein Salehi Surmaghi, Orkideh Dadras, Extraction and Determination of Essential Oil of *Arachis hypogaea* through Supercritical Fluid Extraction Coupled with GC-MS. *Chemical Methodologies* 4(6), 2020, 774-787. DOI: [10.22034/chemm.2020.113872](https://doi.org/10.22034/chemm.2020.113872).