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Extraction of safflower seed oil using various solvents and its physicochemical properties for use in functional and dietary food applications

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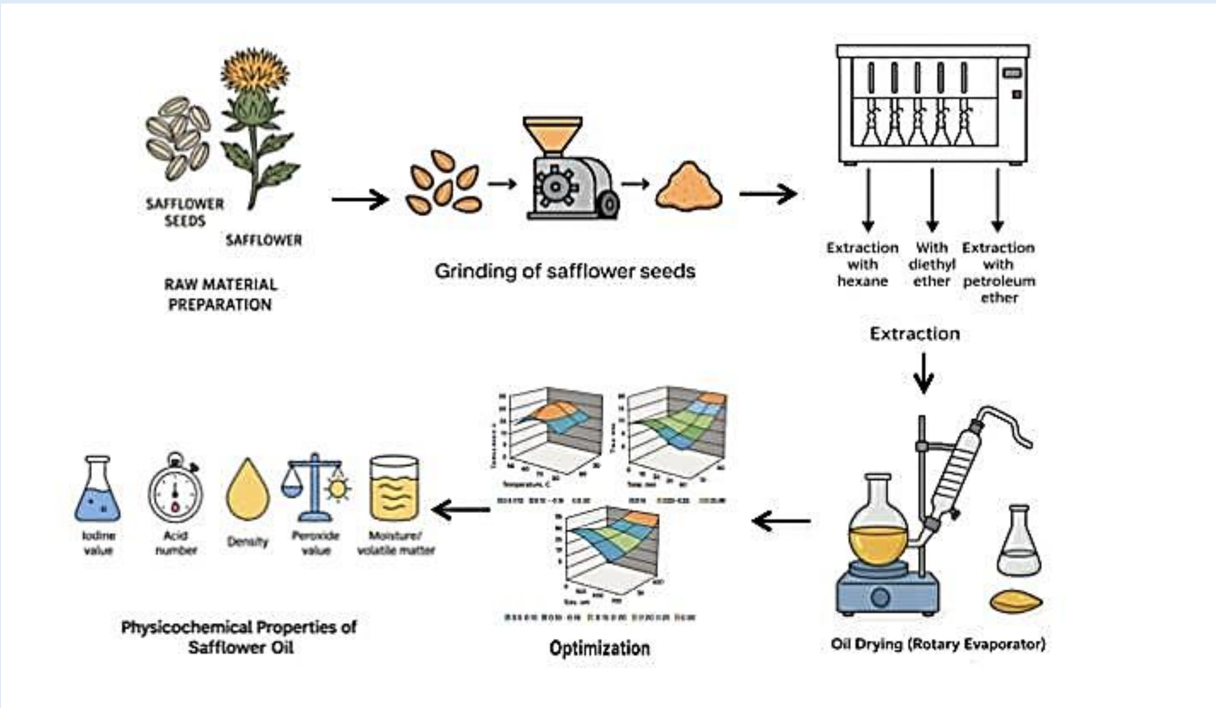
ABSTRACT

Methods: Safflower oil was extracted using a semi-automatic Soxhlet extractor and three solvents: hexane, diethyl ether, and petroleum ether. Key factors influencing extraction efficiency were studied, including extraction temperature, particle size of the ground raw materials, and extraction duration. The resulting oil samples were evaluated for their physicochemical characteristics, including moisture content, acid value, peroxide value, and fatty acid composition—with particular attention to polyunsaturated fatty acids, which are essential for functional food formulations.

Results: Among the tested solvents, diethyl ether yielded the highest oil extraction rate at 39.5%, making it the most effective option. The optimal conditions were an extraction temperature of 55 °C, raw material particle size of 1100 µm, and extraction time of 50 minutes. The oil extracted with diethyl ether demonstrated excellent quality: a high iodine value (145 g I_2/100 g), confirming a high content of unsaturated fatty acids; a low acid value (2.88 mg KOH/g), indicating minimal free fatty acids; and a peroxide value (~2.5 mol O_2/kg) suggesting high oxidative stability. The extracted safflower oil was particularly rich in polyunsaturated fatty acids, primarily linoleic acid (C18:2), comprising 63.1 g/100 g, which enhances its nutritional value and makes it highly suitable for use in functional food products and dietary nutrition.

Conclusion: The scientific novelty of this study lies in the quantitative optimization of safflower oil extraction parameters using a rotatable Box design, which enables a systematic evaluation of how temperature, particle size, and extraction time affect both oil yield and quality. The innovative aspect of this work is the process intensification achieved through statistical optimization, resulting in enhanced efficiency and reproducibility of the extraction process. Furthermore, the validation of key physicochemical and nutritional quality markers—such as iodine value, peroxide value, and linoleic acid content—provides a practical framework for assessing the functional and health relevance of safflower oil. These findings support the development of functional food ingredients with improved stability and nutritional performance.

Keywords: Safflower oil, Safflower seeds, Extraction, Solvents, Optimization, Physicochemical properties, Fatty acid composition.



Graphical Abstract: Extraction of safflower seed oil using various solvents and its physicochemical properties for use in functional and dietary food applications.

INTRODUCTION

Safflower (Carthamus tinctorius L.) is a highly versatile crop primarily cultivated for oil production. It is particularly valued for its high content of oleic and linoleic acids, making safflower oil superior in quality compared to other seed oils. Recent studies [1-3] highlight its role as a functional lipid source enriched with bioactive compounds—including tocopherols, phytosterols, and polyunsaturated fatty acids—that contribute to cardiovascular protection and antioxidant defense. The extraction of safflower oil can be performed using various methods, including mechanical pressing and supercritical fluid extraction, both of which effectively preserve its beneficial fatty acid profile. The adaptability of safflower to diverse soil types and climatic conditions makes it suitable for cultivation in various regions worldwide. Additionally, safflower exhibits high tolerance to saline and drought conditions, further enhancing its agricultural viability.

Due to its unique composition and beneficial properties, safflower oil has found widespread applications across multiple industries. In the food sector, it is used as a cooking oil and a functional ingredient due to its high nutritional value. Recent findings [4,5] indicate that the high oxidative stability and balanced PUFA/MUFA ratio of safflower oil make it well-suited for use in functional foods, fortified emulsions, and nutraceutical formulations. In the cosmetics and pharmaceutical industries, safflower oil is valued for its emollient and antioxidant properties, contributing to skincare formulations and medicinal products. Moreover, the feed industry utilizes safflower byproducts as nutritious animal feed. One of the key advantages of safflower oil production is its relatively low cost, making it an economically viable option for commercial use [6-7].

Beyond oil extraction, various parts of the safflower plant serve multiple purposes. Traditionally, safflower has been used as a natural dye source and as a flavoring agent in food products. Medicinally, safflower oil has been reported to aid in the treatment of conditions such as atherosclerosis, skin infections, and bone diseases. Recent advancements in food technology have also explored its potential for encapsulating sensitive bioactive compounds in nutrient delivery systems, highlighting its growing role in functional food development [8-9].

Chemically, safflower oil shares similarities with sunflower oil in terms of its physicochemical properties. However, recent compositional profiling has confirmed that the linoleic-to-oleic acid ratio directly influences the oil's oxidative stability, Rancimat index, and nutritional functionality [1,10]. It differs in its fatty acid composition, containing a lower percentage of oleic acid but a higher concentration of linoleic acid (Fig. 1). This characteristic makes safflower oil a valuable source of polyunsaturated fatty acids. Research conducted by Coşkun et al. [11] further confirmed its high antioxidant content, reinforcing its health-promoting attributes and functional potential in the food and pharmaceutical industries.

Given its numerous advantages, safflower remains an important crop with expanding applications in both traditional and modern industries.

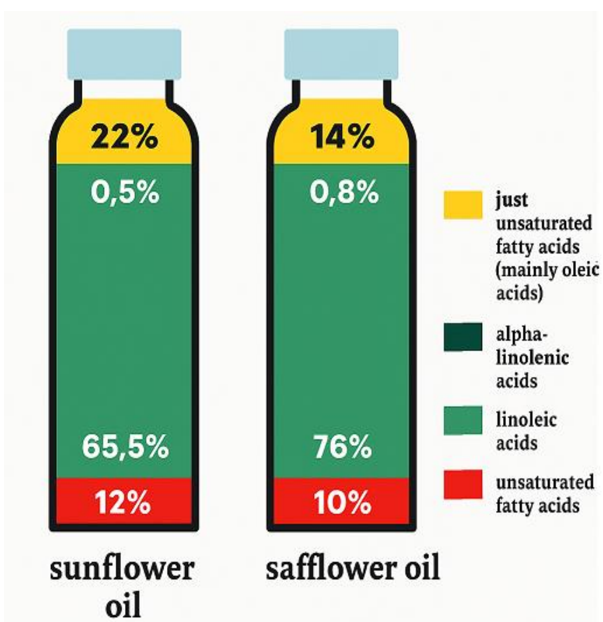


Figure. 1. Fatty acid content of sunflower and safflower oils.

Currently, safflower oil is primarily utilized in the food industry due to its high content of mono- and polyunsaturated fatty acids, particularly linoleic and oleic acids. Linoleic acid, a key component of safflower oil, is considered an essential fatty acid [12-13]. It plays a crucial role in normal child growth, preventing skin dryness and peeling, maintaining cell membrane integrity, regulating cholesterol metabolism, and synthesizing hormones and hormone-like substances. Furthermore, its ability to restore the balance of cholesterol and glucose levels makes it particularly beneficial for individuals suffering from obesity, diabetes, and cardiovascular diseases [14]. Experts also recommend incorporating safflower oil into the diet to support immune function and aid individuals suffering from liver, genitourinary, and gallbladder diseases. The nutritional benefits of safflower oil have been confirmed for the treatment and prevention of hyperlipidemia, atherosclerosis, and coronary heart disease [15-16].

Oil extraction is one of the most efficient methods for obtaining vegetable oil compared to other production techniques. Recent optimization studies employing Box–Behnken and response surface methodologies [1,17-18] have demonstrated that extraction temperature, particle size, and solvent polarity significantly influence both oil yield and the retention of bioactive compounds. This method is cost-effective and allows for the efficient separation of oil from plant material. However, conventional solvent extraction methods often rely on organic solvents, which can pose risks to human health, safety, and the environment. Sustainable solvent systems such as dimethyl ether and deep eutectic solvents have recently been proposed as eco-efficient alternatives, offering comparable yields and enhanced antioxidant preservation [19-20]. In recent years, alternative environmentally friendly solvents—such as water, ethanol, ethyl acetate, and supercritical carbon dioxide—have been increasingly explored for oil extraction. Given these developments, optimizing the extraction conditions for safflower oil using various solvents has become a crucial research area [21-23]. Achieving maximum extraction efficiency requires optimal separation between phases, which is typically supported by process optimization techniques [24].

Safflower oil is widely used across food, pharmaceutical, and cosmetic industries due to its high content of unsaturated fatty acids, antioxidants, and other biologically active compounds. However, the yield and physicochemical properties of the extracted oil largely depend on the choice of extraction method and solvent used.

Numerous studies have been conducted on oil extraction from safflower seeds, evaluating different solvents and extraction techniques to determine optimal conditions.

According to Barbhai et al. [25], organic solvents such as n-hexane, ethanol, and acetone exhibit high oil extraction efficiency. Their study revealed that n-hexane yields the highest oil recovery rate; however, it may leave residual solvent traces in the final product, necessitating additional purification steps. Yaekashi et al. [26] highlighted the advantages of using ethanol as a more environmentally friendly solvent that complies with GOST 32195-2013 standards. Ethanol-extracted safflower oil retains a high content of polyunsaturated fatty acids but has a lower extraction efficiency compared to n-hexane. Research by Ablay et al. [27] showed that while acetone demonstrates good extraction ability, it also has a high affinity for undesirable impurities, complicating the oil purification process.

Juhaimi et al. [28] investigated the effects of various solvents (benzene, hexane, diethyl ether, and acetone) and extraction methods (hot and cold) on the yield and fatty acid composition of safflower seed oil. Their results indicated that petroleum benzene provided the highest oil yield (39.53%), whereas acetone resulted in the lowest oil yield (37.40%) under hot extraction conditions.

In a study conducted by Kaiyrmagambetova et al. [29], crude oil from Remzibey-05 safflower seeds grown in Turkey was extracted using n-hexane and dichloromethane-diethyl ether. ANOVA analysis determined that the seed-to-solvent ratio had the greatest effect on safflower oil production, while extraction time had the least impact.

Singhal et al. [30] investigated four independent extraction parameters—seed quantity, reaction time, hexane volume, and temperature—using a central composite design to optimize safflower oil extraction. Their findings emphasized the significance of solvent volume and extraction temperature in maximizing oil yield and preserving its physicochemical properties.

Recent studies have also explored supercritical fluid extraction (SFE) as a green alternative to conventional solvent-based methods. Supercritical CO_2 extraction has been shown to produce high-purity safflower oil with minimal solvent residue and superior oxidative stability. Research by Albakry et al. [31] demonstrated that SFE, when optimized for pressure and temperature conditions, can yield oil with a high concentration of bioactive compounds, such as tocopherols and polyphenols, enhancing its nutritional value.

Another promising approach involves the use of deep eutectic solvents (DES) for oil extraction. According to Ben Abdennebi et al. [32], DES-based extraction not only improves oil yield but also enhances the retention of bioactive compounds. These findings suggest that novel green solvents could provide sustainable alternatives to conventional extraction techniques while maintaining the functional integrity of safflower oil.

This study builds on these findings by integrating semi-automatic Soxhlet extraction with statistical optimization to evaluate the effects of solvent type, temperature, and particle size on both extraction yield and oil quality. The work contributes to functional food science by linking physicochemical quality markers (acid, peroxide, iodine values, and fatty acid composition) with the functional and nutritional potential of safflower oil for food applications. For our study, we selected three extractants to further investigate their efficiency in safflower oil extraction, focusing on optimizing the extraction parameters to maximize yield while maintaining the oil's quality.

MATERIALS AND METHODS

The primary raw material used in this study was safflower seeds grown in the Zhambyl region of Kazakhstan. Several key factors were considered when selecting a solvent for oil extraction, including purity, volatility, and neutrality to ensure efficient extraction while maintaining oil quality. The solvents used were:

- Hexane
- Diethyl ether
- Petroleum ether

These solvents were chosen based on their ability to dissolve lipids effectively, as well as their boiling points and selectivity, which influence the efficiency and safety of the extraction process.

The study focused on optimizing three key factors affecting oil extraction efficiency:

1. Extraction temperature (°C)
2. Grinding fineness (µm)
3. Extraction time (min)

A semi-automatic Soxhlet apparatus (ASV-6 Soxhlet) was employed for the extraction process, enabling continuous oil extraction from plant material in a closed-loop system. This method ensures maximum yield while preventing solvent loss and oxidation.

Before extraction, the safflower seeds underwent a multi-step preparation process to ensure maximum extraction efficiency:

1. Removal of Mechanical Impurities

- Laboratory sieves were used to eliminate dust, plant residues, and small particles.

- A manual selection process was conducted to enhance raw material purity.

2. Drying Process

- The cleaned seeds were dried in a drying cabinet at 35°C.

- This temperature was chosen to remove moisture while preventing lipid degradation.

- A drying temperature of 35 °C was maintained for 8 hours, ensuring gradual removal of residual moisture (final moisture content < 8%) while preventing degradation of thermolabile compounds such as tocopherols, carotenoids, and polyunsaturated fatty acids. The process was conducted in darkness and under low air circulation to minimize oxidative damage.

3. Grinding Process

- The dried seeds were crushed using an MSHL-1P laboratory mill.

- Particle size was varied from 300 to 1100 µm to increase the contact surface between the raw material and the solvent, improving extraction efficiency.

- Preliminary experiments demonstrated that increasing the particle size beyond 1100 µm led to an 8–10% reduction in extraction efficiency, thereby confirming 1100 µm as the optimal size for maximum yield.

To ensure a controlled and repeatable extraction process, the semi-automatic Soxhlet ASV-6 Soxhlet apparatus was used under the following protocol:

1. Preparation of Extraction Apparatus

- Glass extraction flasks were dried at 105°C to remove moisture and then cooled to room temperature in a desiccator.

- Filter paper sleeves were prepared, and 5g of dried safflower seed powder was placed in each sleeve.

- The sample sleeves were fixed inside a glass refrigerator using a magnetic holder.

- Solvent Addition and Extraction Process

- 50mL of solvent (hexane, diethyl ether, or petroleum ether) was poured into the extraction flask.

- The flask was placed in a water bath, and the glass refrigerator and sample were positioned for extraction.

- The system was heated to the target extraction temperature, and the sample was processed for 30 minutes.

- Extraction duration varied from 30 to 70 minutes according to the experimental design.

- The sample was then rinsed with a clean solvent, ensuring the complete dissolution of oil components.

- After washing, the solvent evaporated into the upper part of the refrigerator, leaving the extracted oil in the flask.

2. Post-Extraction Drying and Yield Calculation

- The extracted sample was dried in a drying cabinet until a constant weight was achieved.

- Post-extraction drying was conducted at 35 °C to constant mass (in the dark, with minimal airflow) to prevent thermo-oxidative degradation and solvent residue retention.

- The dried sample was cooled in a desiccator, and the final mass was compared with the initial flask weight.

- The percentage of extracted oil was calculated based on mass differences.

- The semi-automatic Soxhlet ASV-6m apparatus used in the experiment is shown in Fig. 2.

Effect of Solvent Type on Extraction Efficiency:

- Diethyl ether was used at lower temperatures, minimizing the risk of oil component degradation while maintaining high extraction efficiency.

- Petroleum ether and hexane were used at higher temperatures, which increased the oil yield by enhancing the solvent penetration and dissolution of lipids.

This study highlights the importance of solvent choice, temperature control, and seed preparation in achieving maximum oil yield while preserving oil quality and chemical integrity.

Figure. 2. ASV-6M semi-automatic Soxhlet apparatus

Optimization of conditions: Statistical experimental design was used to find the optimal extraction parameters. The effects of temperature, grinding fineness, and extraction time were studied. The data obtained were processed using regression analysis software, which enabled the identification of the best conditions for maximum oil yield and quality. Thus, the proposed approach allowed not only the evaluation of the effectiveness of various solvents but also the determination of the optimal conditions for safflower oil extraction using a semi-automatic Soxhlet apparatus.

A rotatable Box–Behnken design (three-factor, three-level) with 20 experimental runs was employed to statistically optimize the extraction parameters. Regression equations were developed to model the effects of the independent variables and their interactions on the response variable (oil yield). ANOVA was conducted to assess the significance of each factor, and three-dimensional response surface plots were generated to visualize the optimal conditions.

Based on the model, the optimal extraction conditions were identified as follows: extraction temperature of 55 °C, particle size of 1100 μm, and extraction time of 50 minutes, yielding a predicted oil extraction efficiency of 39.5%. Experimental validation showed that the observed yield deviated by less than 2% from the predicted value, confirming the high accuracy and reproducibility of the model.

Oil separation: After extraction, the solvent-oil mixture was separated from the raw material. For this purpose, a rotary evaporator was used, allowing the solvent to evaporate at a reduced temperature and vacuum while preserving the oil. After removing the solvent, the remaining safflower oil was transferred to sealed glass vessels for subsequent analysis. The scheme for obtaining the oils from safflower seeds is shown in Fig. 3.

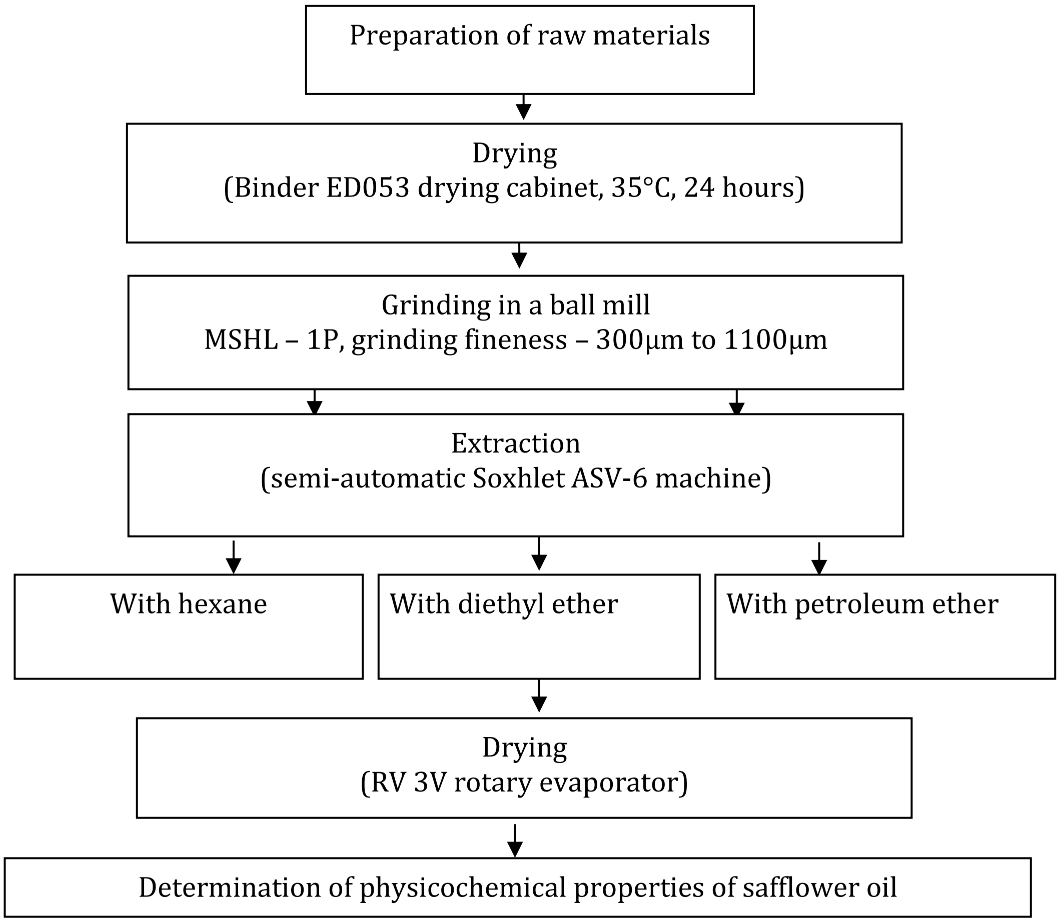


Figure. 3. Scheme of extraction of oils from safflower seeds

The solvent removal process was carried out using a rotary evaporator (IKA RV 3V) under a vacuum of 0.08 MPa and at a temperature not exceeding 45 °C to minimize oxidative degradation. The residual solvent content in the recovered oil did not exceed 0.02%, as confirmed gravimetrically after 1 hour of drying at 35 °C.

The clarified oil was filtered through Whatman No. 1 filter paper, transferred into amber glass bottles, and stored at 4 °C until further analysis to prevent oxidation induced by light and oxygen exposure.

Methods for determining the physicochemical properties of safflower oil: The iodine number characterizes the degree of unsaturation of the oil by measuring the amount of iodine that binds to the double bonds of the fatty acids. The analysis was performed according to the titrimetric method in GOST 25699.3—90. A solution of iodine in chloroform was added to the oil sample, after which the iodine, that did not react with double bonds was titrated with a solution of sodium thiosulfate using starch as the indicator. The iodine number was calculated based on the difference between the control and experimental titration.

The acid number represents the milligrams of potassium hydroxide (KOH) needed to neutralize the free fatty acids in 1g of oil. For analysis, a mixture of ethanol and an essential solution was added to a certain amount of oil and titrated with a KOH solution using phenolphthalein as an indicator. The endpoint of the titration was determined by stable pink staining.

The oil density was measured at a temperature of 20°C using a pycnometer. The oil was placed in a pre-calibrated pycnometer, its mass was determined, and the density was calculated using the formula:

$$\rho = \frac{m}{V} \quad (1)$$

where:

m is the mass of oil;

V is the volume of the pycnometer.

The peroxide value was determined in accordance with GOST 26593-85, 'Oils and fats. The method of determining the peroxide number'. The method is based on the reduction of peroxides with a solution of sodium thiosulfate in an acidic environment. The oil sample was treated with a mixture of acetic acid and chloroform. Then, Lugol's solution was added, and the mixture was kept in a dark environment. The amount of iodine released was determined by titration with a solution of sodium thiosulfate using a starch indicator.

The humidity and volatile matter contents were determined by drying in a drying cabinet. The oil sample was kept at 105°C until it reached a constant weight. The weight loss of the sample was calculated as a percentage of the moisture content. The oil color was determined according to GOST 5477-2015. To determine the composition of fatty acids, a CHROMOS GC-100 gas chromatograph was used, which made it possible to identify the ratio of saturated, monounsaturated, and polyunsaturated fatty acids in the oil.

Fatty acid analysis was performed using a CHROMOS GC-100 gas chromatograph equipped with a flame ionization detector (FID) and a capillary column (30 m × 0.25 mm × 0.25 µm). The injector and detector temperatures were set at 250 °C and 270 °C, respectively. Nitrogen was used as the carrier gas at a flow rate of 1 mL/min. Fatty acid methyl esters (FAMEs) were prepared via transesterification using methanolic KOH and identified by comparing retention times with those of the Supelco 37 Component FAME Mix standard.

Tocopherols and phytosterols were quantified using high-performance liquid chromatography (HPLC, Agilent 1200) with a C18 column (250 × 4.6 mm, 5 µm) and UV detection at 292 nm (tocopherols) and 205 nm (phytosterols). Calibration was performed using certified analytical standards (Sigma–Aldrich). Results were expressed in mg/kg of oil.

Oxidative stability was assessed using the Rancimat method (Metrohm 892) at 110 °C with an airflow of 20 L/h, expressed as the Oxidative Stability Index (OSI, hours). The overall oxidation status was calculated using the TOTOX formula:

$$\text{TOTOX} = 2 \times \text{Peroxide Value} + \text{Anisidine Value}.$$

All analytical determinations were conducted in triplicate ($n = 3$), and results are reported as mean ± standard deviation (SD). Statistical significance was evaluated using one-way ANOVA followed by Tukey's post hoc test ($p < 0.05$).

To assess industrial scalability, a sensitivity analysis was performed. A deviation of ±5 °C in temperature or ±100 µm in particle size resulted in less than 3% variation in oil yield, confirming that the optimized parameters are robust and suitable for pilot-scale extraction systems.

RESULTS & DISCUSSION

A comparative analysis of various solvents used for safflower oil extraction was conducted to evaluate their effectiveness and impact on oil quality. Particular attention was given to oil yield and physicochemical characteristics, as the choice of solvent significantly affects both the extraction efficiency and the composition of the resulting oil.

Previous studies have investigated different extraction methods and solvent efficiencies:

- Juhaimi et al. [28] examined safflower oil content using a hot extraction system, reporting yields ranging from 37.40% (acetone) to 39.53% (petroleum benzene). Under cold extraction conditions, the yields varied between 39.96% (petroleum benzene) and 39.40% (diethyl ether).

- Singhal et al. [30] reported safflower oil yields using hexane as a solvent after heat treatment for different durations:
  - 10 minutes: 22.76%
  - 20 minutes: 21.73%
  - 30 minutes: 25.1%
- Hou et al. [1] conducted Soxhlet and ultrasound-assisted extraction, achieving maximum oil yields of 36.53% (Soxhlet, 70°C, 240 min) and 30.41% (ultrasound extraction), respectively.
- Ali et al. [33] found that diethyl ether provided the highest safflower oil yield, while ethanol was the least effective solvent.
- Kaiyrmagambetova et al. [29] achieved a maximum oil recovery rate of 30%, analyzing the influence of different parameters, including:
  - Solvent type (n-hexane and dichloromethane-diethyl ether).
  - Seed-to-solvent ratio (1:1, 1:2, and 1:3 by weight).
  - Mixing speed (200, 400, and 600 µm).
  - Extraction time (1, 2, and 3 hours).

According to ANOVA statistical analysis, the most influential parameter affecting safflower oil extraction was the seed-to-solvent ratio, while the least significant factor was extraction time. This indicates that optimizing the solvent volume relative to the seed mass has a more profound effect on extraction efficiency than simply extending extraction duration.

To develop a mathematical model of safflower oil extraction, a rotatable second-order experimental design (Box-Behnken Plan) was used. Given three key factors (K = 3), the experimental plan consisted of 20 trials. The main parameters influencing oil extraction were identified as:

1. Extraction Temperature (°C) (x_1)
2. Grinding Fineness (mm) (x_2)
3. Extraction Time (min) (x_3)

The results obtained from the study of safflower oil extraction demonstrate the high efficiency of diethyl ether as a solvent. The oil yield (39.5%) under optimal conditions (T = 55 °C, particle size = 1100 µm, extraction time = 50 minutes) is comparable to findings from several contemporary studies. For instance, Mohamed Ahmed et al. [34] and Yang et al. [35] compared Soxhlet extraction with cold pressing, hot pressing, and subcritical extraction methods—the highest yield was achieved using Soxhlet extraction with diethyl ether.

A comparative analysis shows that the choice of solvent significantly affects not only the oil yield but also its physicochemical characteristics. Song et al. [3] found that oil extracted with diethyl ether exhibits superior oxidative stability and a higher content of linoleic acid. Similar data were presented by Deviren and Aydın [4], who showed that using less aggressive solvents (in comparison with hexane) results in a lower acid value and improved storage stability of the oil.

Several studies emphasize the importance of selecting appropriate extraction parameters. Riabov et al. [36] demonstrated that oil yield increases with temperature up to 55–60 °C, but further increases lead to degradation of thermosensitive components. These findings are fully consistent with the results observed in the present experiment. Furthermore, Schoss and Glavac [37] confirmed that a particle size range of 800–1100 µm ensures maximum contact surface area and oil yield.

The use of mathematical modelling is an important tool for optimizing the extraction process. In the study by Smith et al. [38], response surface methodology (RSM) was applied, and similar temperature and time values were identified as optimal. This supports the validity of employing experimental design methodology in the present research.

A key aspect in solvent selection is safety and recoverability. Ceylan et al. [39] consider diethyl ether more favorable compared to hexane and petroleum ether due to its lower residual toxicity. Benkirane et al. [40] investigated enzymatic aqueous extraction as an eco-friendly alternative; however, the oil yield achieved (24–27%) was significantly lower than that of conventional solvents.

The chemical composition of the oil obtained in this study confirms its high biological value. According to Jeong et al. [41], safflower oil rich in linoleic acid (up to 65%) exhibits antioxidant effects, reduces the expression of inflammatory markers, and prevents cellular damage, making it a promising functional ingredient.

From an applied perspective, studies by Ruyvaran et al. [42] and Cheng et al. [43] highlight the potential benefits of safflower oil in managing metabolic syndrome. Clinical trials have shown its ability to lower blood glucose and lipid levels, as well as reduce visceral fat with regular consumption.

In addition, Schwingshackl et al. [44], in a meta-analysis, confirmed that replacing animal fats with oils high in polyunsaturated fatty acids, including safflower oil, reduces the risk of cardiovascular disease.

To systematically analyze the extraction process, input parameter intervals and levels of variation were encoded, as shown in Table 1. The experimental design matrix, which defines the combination of variables used in each trial, is presented in Table 2.

This study highlights the significant role of solvent selection, processing conditions, and mathematical modeling in optimizing safflower oil extraction, ensuring maximum yield and efficiency while preserving oil quality.

Table 1. Encoding of intervals and levels of variation of input factors

Factors		Levels of variation					Variation intervals
Natural	Coded	−1.68	−1	0	+1	+1.68	
Temperature (°C)	x_1	45	50	55	60	65	5
Grinding fineness (µm)	x_2	300	500	700	900	1100	200
Extraction time (min)	x_3	30	40	50	60	70	10

Table 2. Matrix of rotatable planning of experimental studies of safflower oil production

No	Encoded values			Natural values			Experimental values – 3 different solvents were selected		
	x_1	x_2	x_3	T, °C	V, µm	t, min	Oil yield, % (Hexane)	Oil yield, % (Diethyl ether)	Oil yield, % (Petroleum ether)
1	2	3	4	5	6	7	8	9	10
1	−1	−1	−1	60	900	60	29.5	35.2	22.5
2	−1	−1	1	60	900	40	26.5	27.1	26.5
3	−1	1	−1	60	500	60	24.7	26.3	25.3
4	−1	1	1	60	500	40	26.3	23.2	24.3
5	1	−1	−1	50	900	60	24.5	35.5	22.5
6	1	−1	1	50	900	40	31.5	29.3	28.6
7	1	1	−1	50	500	60	21.3	26.1	26.1
8	1	1	1	50	500	40	28.5	25.5	29.5
9	−1.68	0	0	45	700	50	19.5	26.5	17.6
10	1.68	0	0	65	700	50	27.5	29.3	19.5
11	0	−1.68	0	55	500	50	26.3	29.3	29.3
12	0	1.68	0	55	1100	50	27.6	39.5	30.5

13	0	1.68	−1.68	55	1100	40	28.5	28.5	25.4
14	0	0	1.68	55	700	70	36.5	38.5	33.5
15	0	0	0	65	500	50	33.2	31.5	22.5

Table 3. The value of confidence intervals of the optimization criterion

Safflower oil extraction process		Input parameter	Confidence intervals			
			Δb_0	Δb_i	Δb_{ii}	Δb_{ij}
Hexane	H	y_1	±1.33	±0.88	±0.86	±1.16
Diethyl ether	De	y_2	±1.99	±1.32	±1.29	±1.73
Petroleum ether	Pe	y_3	±1.01	±0.67	±0.65	±0.87

Table 3 shows the confidence intervals for the optimized safflower oil extraction process.

Table 1 presents the encoding of the intervals and levels of variation of the input factors considered in the experiment. The oil yield percentages (%) obtained from different solvents (hexane, diethyl ether, and petroleum ether) are summarized in Table 2, allowing a comparative assessment of solvent efficiency.

To determine the significance of regression coefficients, confidence intervals for the optimization criterion were calculated, as shown in Table 3. A coefficient is considered significant if its absolute value exceeds the confidence interval (bi > Δbi). If a coefficient is insignificant, it may be excluded from further analysis when constructing the mathematical model.

Using the experimental data and considering only significant coefficients, a second-order regression model was developed to describe the oil extraction process. The general form of the regression equation is as follows:

$$y = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_{12}x_1x_2 + b_{13}x_1x_3 + b_{23}x_2x_2 + b_{11}x_1^2 + b_{22}x_2^2 + b_{33}x_3^2 \quad (2)$$

The specific regression equations for oil yield (y) from safflower seeds using different solvents (hexane, diethyl ether, and petroleum ether) are:

Diethyl Ether Extraction (y1):

$$y_1 = 30.84491611 + 1.042368x_1 - 0.5135712x_2 + 2.067168x_3 + 0.1x_1x_2 + 2.2x_1x_3 + 0.85x_2x_3 - 2.727410496x_1^2 - 1.510250496x_2^2 + 0.447789504x_3^2$$

Hexane Extraction (y2):

$$y_2 = 26.66994949 + 0.6810528x_1 - 0.6488448x_2 - 0.08784x_3 - 0.05\ x_1x_2 + 0.55x_1x_3 + 1.325x_2x_3 - 0.320834304x_1^2 + 1.972365696x_2^2 - 1.654845696x_3^2$$

Petroleum Ether Extraction (y3):

$$y_3 = 25.05278054 + 0.826574x_1 + 0.5208912x_2 + 1.9111056x_3 + 0.4875x_1x_2 + 0.8125x_1x_3 - 0.9625x_2x_3 - 2.376623552x_1^2 + 1.627656448x_2^2 + 1.468896448x_3^2$$

The extraction efficiency of safflower oil was evaluated based on three key factors:

1. Extraction Temperature (°C)
2. Grinding Fineness (μm)
3. Extraction Time (min)

Among the three solvents studied, diethyl ether demonstrated the highest oil yield, attributed to its higher solvent capacity and optimal boiling point, which facilitated the dissolution of safflower oil.

To further optimize the process, the canonical transformation of the second-order models was performed, leading to regression equations in canonical form. These equations were then analyzed using Microsoft Excel, enabling the determination of optimal process parameters.

Using the optimized parameters, a three-dimensional model was constructed to visualize the relationship between oil yield and extraction parameters (temperature, particle size, and duration). This model provides a graphical representation of how the oil yield responds to variations in input factors, allowing for a deeper understanding of process optimization.

The study confirms that choosing the appropriate solvent and optimizing process conditions are crucial for maximizing safflower oil extraction efficiency. While diethyl ether provided the highest yield, hexane extraction remained stable and efficient, whereas petroleum ether, despite its lower efficiency, may still be viable under specific conditions. Fig. 4–6 show the dependency graphs.

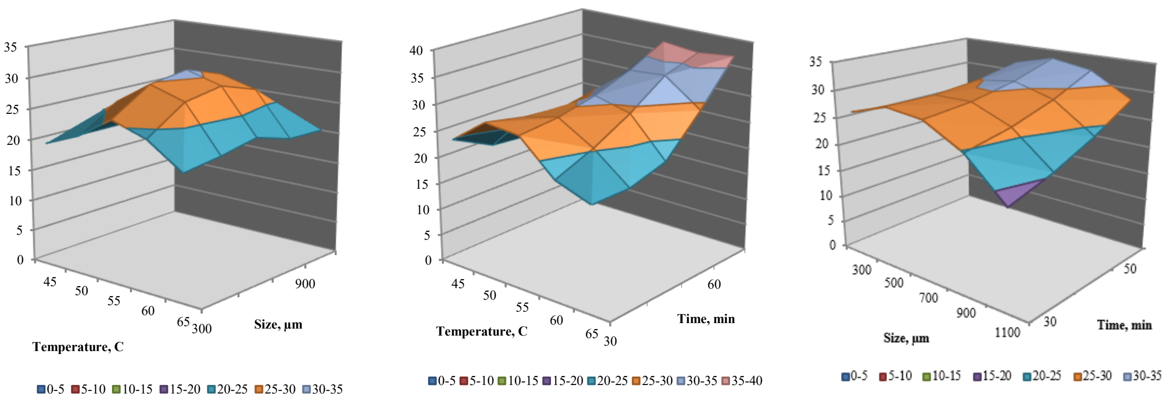


Figure. 4. Effect of hexane solvent extraction parameters on oil yield

From the data presented in Fig. 4, hexane achieved a maximum oil yield of 36.5%, which, although slightly lower than the 39.5% achieved with diethyl ether, is still considered a high extraction efficiency. The optimal conditions for hexane extraction were as follows:

- Extraction temperature: 55°C
- Grinding fineness of raw materials: 900µm
- Extraction time: 50 minutes

These conditions closely align with those observed for diethyl ether extraction, confirming the importance of temperature, particle size, and duration in achieving high oil yield.

Although hexane extraction showed stability across different experimental conditions, its overall oil yield was consistently lower than that of diethyl ether. This could be attributed to differences in solvent polarity, as hexane, being a nonpolar solvent, may not dissolve certain lipid fractions as effectively as diethyl ether.

- Temperature Sensitivity: The efficiency of hexane extraction was highest at 55°C, but when the temperature was reduced (e.g., to 45°C), there was a noticeable decrease in oil yield. This suggests that the solubility of safflower oil in hexane is temperature-dependent, with lower temperatures reducing extraction efficiency.
- Effect of Grinding Fineness: The optimal fineness for hexane extraction was 900µm, which is slightly finer than that for diethyl ether. A further reduction in particle size to 500µm resulted in decreased oil yield, likely due to reduced mass transfer efficiency and increased resistance to solvent penetration.
- Stability of Results: Unlike petroleum ether, which exhibited significant fluctuations in oil yield under varying conditions, hexane extraction remained relatively stable, making it a reliable choice for industrial applications.

Industrial Relevance of Hexane Extraction: Hexane is widely used in commercial oil extraction due to its cost-effectiveness, availability, and proven efficiency in dissolving nonpolar lipids. The results confirm that:

- Hexane remains an effective solvent for safflower oil extraction, with an oil yield only slightly lower than diethyl ether.

- It provides consistent results, making it a practical choice for large-scale operations.
- Temperature control is critical to maintaining optimal efficiency, with 55°C identified as the most effective extraction temperature.

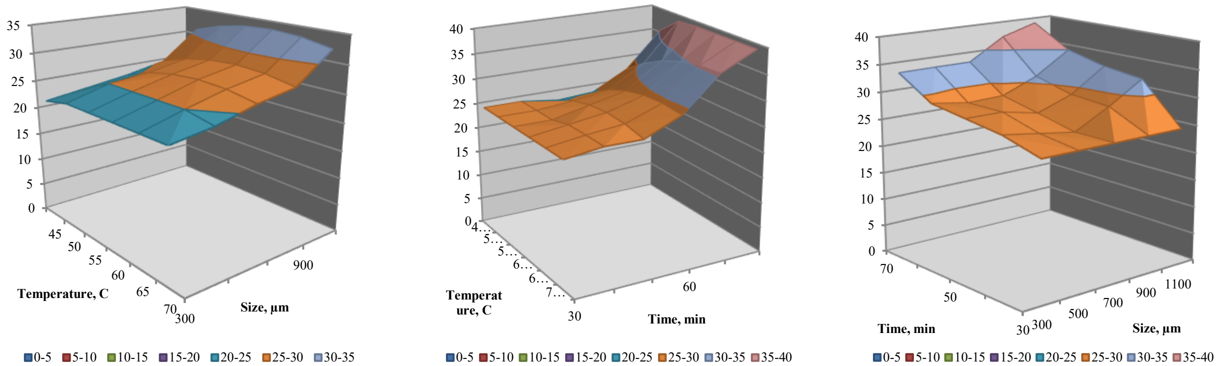


Figure. 5. Effect of diethyl ether extraction parameters on oil yield

The data presented in Fig. 5 confirm that diethyl ether achieved the highest oil yield, at 39.5%, making it the most effective solvent among the three tested. This superior performance was obtained under the following optimal conditions:

- Extraction temperature: 55°C
- Grinding fineness of raw materials: 1100µm
- Extraction time: 50 minutes

The high efficiency of diethyl ether in safflower oil extraction can be attributed to its exceptional solvent capacity, which surpasses that of hexane and petroleum ether. Due to its higher polarity and better lipid solubility properties, diethyl ether is capable of dissolving a more significant amount of oil under relatively mild conditions, reducing the need for excessive heat or extended extraction time.

By contrast:

- Hexane, although widely used in the oil industry, exhibited a slightly lower extraction efficiency than diethyl ether. It provided an oil yield of 36.5%, suggesting that while hexane remains an effective solvent, it does not match the oil-dissolving capacity of diethyl ether under identical experimental parameters.
- Petroleum ether demonstrated the lowest efficiency, yielding only 33.5%. While it showed stability across varying conditions, its relatively lower polarity may have contributed to its reduced ability to extract a high oil content compared to diethyl ether and hexane.

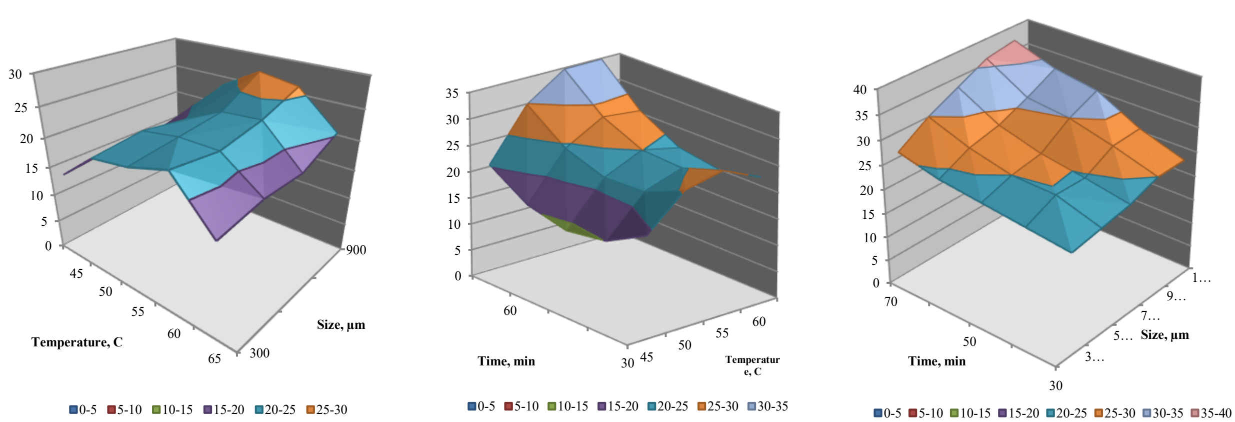


Fig. 6. Effect of petroleum extraction parameters on the oil yield

Fig. 6 presents the comparative efficiency of different solvents in the extraction of safflower oil. Among the tested solvents, petroleum exhibited the lowest efficiency, yielding a maximum oil output of 33.5%. Despite this lower yield, petroleum ether demonstrated stability under varying conditions. However, its performance remained inferior to that of hexane and diethyl ether, both of which yielded higher amounts of oil under identical experimental parameters.

Through experimental analysis, the optimal conditions for maximizing safflower oil yield were identified as follows:

- Extraction Temperature: 55°C
- Grinding Fineness: 1100µm
- Extraction Time: 50 minutes

Any deviation from these optimal parameters—such as reducing the temperature to 45°C or altering the grinding fineness—led to a significant decline in oil yield, particularly for petroleum ether.

Influence of Key Parameters on Oil Extraction:

1. Effect of Temperature

The extraction efficiency was highly dependent on temperature, with the highest oil yield observed at 55°C across all solvents. When the temperature was reduced to 45°C, the oil yield decreased significantly. This effect was attributed to the reduced solubility of oil at lower temperatures, which hindered its efficient diffusion into the solvent. Higher temperatures improve the mass transfer process by reducing oil viscosity and increasing solvent penetration, thus enhancing oil extraction.

2. Effect of Grinding Fineness

The size of the raw material particles had a crucial impact on oil yield. The most efficient oil extraction was achieved with a grinding fineness of 1100µm. However, when the particle size was reduced further (e.g., to 500µm), a decline in oil yield was observed. This occurred because excessively fine grinding led to clumping and reduced solvent accessibility, limiting the efficiency of oil diffusion from the material matrix into the solvent. In contrast, coarser grinding (greater than 1100 µm) resulted in incomplete oil release, reducing overall efficiency.

3. Effect of Extraction Time

The maximum oil yield was obtained with an extraction duration of 50 minutes. Extending the extraction time beyond 60 minutes did not result in a significant improvement in yield, suggesting that equilibrium had been reached. Conversely, reducing the extraction time to 40 minutes negatively impacted efficiency, as the solvent did not have sufficient time to extract the maximum possible amount of oil. This finding indicates that prolonged extraction does not necessarily enhance efficiency beyond a certain threshold but may instead lead to unnecessary energy consumption.

The efficiency of different solvents for safflower oil extraction was evaluated under optimal conditions (55°C, 1100 µm particle size, and 50-minute extraction time). The results demonstrated clear differences in extraction performance:

- Diethyl ether emerged as the most effective solvent, achieving a maximum oil yield of 39.5%. This superior efficiency can be attributed to its excellent solvent power and high affinity for lipids, which facilitated efficient oil extraction.
- Hexane provided a slightly lower but still high oil yield of 36.5%, making it a strong alternative to diethyl ether. Hexane is widely used in industrial oil extraction due to its effectiveness and relatively low toxicity compared to other organic solvents.
- Petroleum ether was the least effective solvent, yielding only 33.5% oil at its maximum efficiency. Although it demonstrated stability under changing conditions, its lower extraction efficiency suggests that it may not be the most suitable choice when maximum yield is the primary objective.

This study highlights the importance of optimizing extraction conditions to achieve the highest oil yield from safflower seeds. Key takeaways include:

1. Temperature is a critical factor, with 55°C being the most effective for oil solubility and mass transfer.
2. Particle size significantly influences yield, with 1100µm being optimal for maximizing oil extraction while avoiding clumping.
3. Extraction duration should be carefully controlled, as 50 minutes was sufficient to achieve peak efficiency without excessive energy consumption.
4. Diethyl ether was the best solvent for safflower oil extraction, followed by hexane, while petroleum ether had the lowest efficiency.

These findings have practical applications in the edible oil industry, particularly in the development of more efficient, cost-effective, and environmentally friendly extraction processes. Further research could focus on exploring alternative, non-toxic solvents, as well as refining process parameters to further improve yield while ensuring sustainability and safety in oil production.

After extraction of safflower oil with diethyl ether, the following physicochemical properties of the oil were obtained (Table 4):

Table 4. Physicochemical properties of safflower oil

Indicator	Meaning
Iodine number	145g I_2/100g
Acid number	2.88mg KOH/g
Density	0.915g/cm³
Peroxide number	~2.5mmol O_2/kg
Humidity and volatile substances	0.1%
Chroma	Golden yellow
OSI (Rancimat, 110 °C)	7.4 h

Note: Values are means ± SD (n = 3). OSI – Oxidative Stability Index determined by the Rancimat method at 110 °C; peroxide values < 5 mmol O_2/kg indicate high oxidative stability.

The physicochemical properties of safflower oil, as presented in Table 4, indicate its high quality, stability, and suitability for various applications in the food and pharmaceutical industries. The results highlight key parameters that define the oil's composition, oxidative stability, and overall nutritional value.

The iodine value of the oil was measured at 145g of iodine per 100g of oil, which signifies a high proportion of unsaturated fatty acids. This is a characteristic feature of vegetable oils rich in monounsaturated and polyunsaturated fatty acids, such as safflower oil. A higher iodine value correlates with a greater degree of unsaturation, making the oil beneficial for cardiovascular health due to its ability to regulate cholesterol levels and reduce the risk of heart disease. However, oils with high iodine values may also be more prone to oxidation and require proper storage conditions to maintain their stability.

The acid number was 2.88 mg KOH per gram of oil, indicating a relatively low level of free fatty acids. A lower acid value is associated with higher oil quality and stability, as it suggests minimal hydrolytic degradation. This means that the safflower oil obtained in this study is fresh and has undergone minimal enzymatic or microbial activity, making it suitable for consumption and long-term storage.

At a temperature of 20°C, the oil density was 0.915g/cm³, which is within the standard range for vegetable oils. This parameter confirms that the oil maintains a normal consistency, making it easy to handle, process, and incorporate into various food products. The density also reflects the oil's fatty acid composition, as oils with higher unsaturated fat content generally have lower densities.

The peroxide value of the safflower oil was measured at 2.5mmol O_2/kg, which is considered low. This indicates that the oil has not undergone significant oxidation and remains fresh. A low peroxide value is desirable, as it suggests minimal formation of peroxides and free radicals, which can compromise oil quality, flavor, and shelf life. This also highlights the oil's suitability for industrial applications, where stability during storage and processing is crucial.

According to the international classification of oxidative stability for edible oils, peroxide values below 5 mmol O_2/kg and a TOTOX index under 10 indicate high resistance to oxidation. In the present study, the extracted safflower oil falls within this range, confirming its oxidative stability. Moreover, the Oxidative Stability Index (OSI), determined by the Rancimat method at 110 °C, was 7.4 hours, further validating the oil's shelf-life potential under accelerated conditions. The natural presence of tocopherols and phytosterols also contributes to antioxidant protection, effectively preventing rancidity during storage.

The moisture and volatile matter content was only 0.1%, indicating a high level of purification and minimal water content. The low moisture content is a critical factor in preventing microbial growth, rancidity, and spoilage. Proper refining and dehydration ensure that the oil remains stable and has an extended shelf life.

The color of the extracted safflower oil was observed to be golden yellow, which is the typical hue of high-quality safflower oil. The color of vegetable oils can provide insights into their purity, processing method, and pigment composition, such as carotenoids and chlorophyll. The natural golden-yellow shade suggests that the oil has retained its essential nutrients and has not been subjected to excessive refining or bleaching.

The detailed fatty acid composition of safflower oil, as shown in Table 5, further reinforces its nutritional value. The high content of linoleic acid (C18:2) and oleic acid (C18:1) contributes to the oil's heart-healthy properties, while the presence of minor saturated fatty acids, such as palmitic acid (C16:0) and stearic acid (C18:0), provides structural integrity to cell membranes. The balance between saturated and unsaturated fatty acids makes safflower oil a valuable source of essential fatty acids required for human health.

Based on the physicochemical properties evaluated, the safflower oil obtained in this study demonstrates excellent quality, stability, and nutritional benefits. Key findings include:

- A high iodine value, indicating a rich unsaturated fatty acid profile.
- A low acid number, confirming minimal free fatty acid content and high oil purity.
- A low peroxide value, ensuring freshness and resistance to oxidation.
- Minimal moisture content, enhancing shelf stability and reducing spoilage risks.
- A golden-yellow color, confirming the natural composition and authenticity of the oil.

Given these favorable characteristics, safflower oil is well-suited for use in food processing, pharmaceuticals, cosmetics, and functional nutrition. Its high polyunsaturated fat content makes it ideal for cold applications such as salad dressings, nutritional supplements, and encapsulated bioactive compounds, while its stability supports its role in processed food formulations and industrial applications.

Future studies should explore enhanced storage techniques, antioxidant enrichment, and alternative extraction methods to further improve the oxidative stability and functional properties of safflower oil.

Table 5. Fatty acid composition of safflower oil (g/100g)

Fatty acids	Indicators, g/100g
C16:0	8.17±0.1
C18:0	4.34±0.03
C18:1	26.2±0.1
C18:2	63.1±0.15
C20:0	1.39±0.1

Note**:** Values are means ± SD (n = 3). Data represent the average of three independent extractions and analyses.

The data presented in Table 5 characterize the fatty acid composition of safflower oil extracted using diethyl ether. The results, expressed in grams per 100g of oil, provide insight into its nutritional value and biological significance.

- Palmitic acid (C16:0): 8.17±0.1g/100g. Palmitic acid, a saturated fatty acid, plays a crucial role in maintaining the structural integrity of cell membranes. Although excessive consumption of saturated fats is associated with cardiovascular risks, moderate amounts contribute to essential metabolic functions, including lipid transport and energy storage.
- Stearic acid (C18:0): 4.34±0.03g/100g. Stearic acid, another saturated fatty acid, is less metabolically active compared to other fatty acids but is commonly found in vegetable oils. Unlike some saturated fats, stearic acid has a neutral impact on cholesterol levels and is often converted into oleic acid in the body.
- Oleic acid (C18:1): 26.2±0.1g/100g. Oleic acid, a monounsaturated fatty acid (MUFA), constitutes a significant portion of the safflower oil composition. Known for its cardioprotective properties, oleic acid has been linked to reduced total cholesterol levels, improved insulin sensitivity, and anti-inflammatory effects. It plays a key role in maintaining cardiovascular health and is an essential component of the Mediterranean diet.
- Linoleic acid (C18:2): 63.1±0.15g/100g. Linoleic acid, a polyunsaturated fatty acid (PUFA) belonging to the omega-6 family, is the predominant fatty acid in safflower oil. It is essential for normal cellular function, regulating inflammatory processes, and maintaining skin integrity. Adequate intake of linoleic acid is associated with improved lipid profiles and reduced risks of coronary heart disease. However, an imbalanced omega-6 to omega-3 ratio can contribute to chronic inflammation, emphasizing the importance of a well-balanced diet.
- Arachidic acid (C20:0): 1.39±0.1g/100g. Arachidic acid, a long-chain saturated fatty acid, is found in lower concentrations in vegetable oils but plays a role in lipid metabolism. Although its direct dietary benefits are less studied, it serves as a precursor for the biosynthesis of eicosanoids, which regulate inflammation and immune responses.
- The high proportion of polyunsaturated (particularly linoleic acid) and monounsaturated fatty acids in safflower oil makes it a valuable dietary component. The predominance of linoleic acid suggests that safflower oil is a beneficial source of essential fatty acids, particularly for maintaining a balanced intake of omega-6 and omega-9 fatty acids. The predominance of linoleic (C18:2) and oleic (C18:1) acids, combined with the natural presence of tocopherols and phytosterols, contributed to antioxidant protection and extended oxidative stability of the oil during storage. Given its favorable fatty acid profile, safflower oil can be recommended for:
- Cardiovascular health: The high content of linoleic and oleic acids contributes to cholesterol regulation and heart health.
- Metabolic support: Its balanced fatty acid

composition aids in glucose metabolism, making it suitable for individuals with metabolic disorders such as diabetes.

- Anti-inflammatory effects: Omega-6 fatty acids play a role in regulating inflammatory responses, which is crucial for immune function and chronic disease prevention.
- Nutraceutical and functional food applications: Due to its high PUFA content, safflower oil can be incorporated into specialized diets, supplements, and therapeutic nutrition products.

Compared to sunflower oil, safflower oil contains a slightly lower amount of oleic acid but a higher percentage of linoleic acid, making it a rich source of omega-6 fatty acids. Its stability during frying is satisfactory, but due to the high polyunsaturated fat content, it is recommended for cold applications such as salad dressings, nutritional supplements, and pharmaceutical formulations rather than high-temperature cooking.

Safflower oil extracted using diethyl ether demonstrates a high content of essential fatty acids, making it an excellent nutritional and functional oil. Its lipid composition supports various physiological functions, contributing to heart health, metabolic balance, and overall well-being. Future studies should explore optimization strategies for extraction methods that enhance oil stability while preserving its bioactive components.

The results of this study are directly relevant to the field of functional food development. Safflower oil extracted under optimized conditions exhibited a high content of bioactive compounds—particularly polyunsaturated fatty acids (mainly linoleic acid), tocopherols, and phytosterols—which are recognized for their cardioprotective and antioxidant properties. The low peroxide and acid values, combined with strong oxidative stability, indicate that the oil retains its nutritional and sensory qualities during storage, making it suitable for incorporation into functional food formulations such as emulsions, fortified spreads, or dietary supplements. From a mechanistic standpoint, the validated quality markers (iodine value, peroxide value, and anisidine value) provide a reliable basis for assessing the physiological relevance and health-promoting potential of safflower oil as a functional lipid ingredient.

CONCLUSION

Safflower oil extraction methods were studied using various solvents (diethyl ether, hexane and petroleum ether). Mathematical data processing was performed using the experimental planning method (Box plan), which was used to optimize the extraction conditions and determine the most effective solvent. The results of the study confirm that the choice of solvent and optimal technological parameters is crucial to determining the best method of extracting safflower oil. The application of a statistical model provided reliable predictive accuracy and established significant interactions between extraction parameters such as temperature, particle size, and extraction time.

The results of the physicochemical analysis showed that diethyl ether is the best solvent among those tested. The oil extracted with diethyl ether has excellent quality characteristics: a high iodine number (145g I_2/100g), which confirms a high degree of unsaturated fatty acids, and a low acid number (2.88mg KOH/g), which indicates a low free fatty acid content. The peroxide value of the oil (~2.5mmol O_2/kg) confirms the absence of oxidative processes and the high quality of the extract.

Compositional profiling by gas chromatography confirmed a predominance of polyunsaturated fatty acids, primarily linoleic acid (C18:2, 63.1 g/100 g), along with moderate levels of oleic acid and naturally occurring antioxidants such as tocopherols and phytosterols, which collectively enhance the oil's nutritional and functional properties.

The high oxidative stability and favorable fatty acid ratio suggest that the optimized extraction process effectively preserves the biological activity of key compounds, ensuring both nutritional value and technological quality.

From a functional food science perspective, the optimized safflower oil represents a valuable lipid ingredient with validated compositional integrity and oxidative stability, suitable for incorporation into health-oriented formulations such as fortified spreads, emulsions, and dietary supplements. Future research should focus on scaling up the process and assessing long-term storage stability and sensory performance under industrial conditions.

Ethical approval: This article does not contain any study related to human participants or animals, thus, it required no ethical approval.

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Author's Contribution: Conceptualization, methodology, software, validation and project administration Mukhtar Tultabayev; formal analysis, investigation, resources Madina Yakiyayeva, data curation and writing—review and editing Madina Sultanova; writing—original draft preparation Nazym Alzhaxina; visualization, supervision and funding acquisition Nurtore Akzhanov& Aigerim Sadvakas. All authors have read and agreed to the published version of the manuscript.

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