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OPTIMIZATION OF ULTRASOUND-ASSISTED EXTRACTION AND PHYSICOCHEMICAL CHARACTERIZATION OF PEONY SEED OIL FOR FUNCTIONAL FOOD APPLICATIONS

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Abstract
Peony seed oil (PSO) is a promising source of high-value edible oil, rich in polyunsaturated fatty acids and bioactive compounds. However, its industrial-scale extraction remains suboptimal. In this study, ultrasound-assisted extraction (UAE) was optimized using a single-factor experimental design followed by response surface methodology (RSM), employing Design-Expert® software for process modeling and regression analysis. The optimal extraction conditions 45 °C, 1.5 h, and a solvent-to-solid ratio of 8:1 yielded a maximum oil recovery of 22.98%. The physicochemical characterization revealed a favorable fatty acid composition dominated by α-linolenic acid (43.1%), linoleic acid (27.4%), and oleic acid (24.3%). Antioxidant activity was evaluated through DPPH, ABTS, and FRAPS assays, demonstrated over 85% radical scavenging capacity, indicating significant oxidative stability and health-promoting potential. Further analysis showed conjugated diene and triene values of 3.52 and 3.34, respectively, and high β-carotene content (2,267 mg/kg). Stability assessments under thermal stress revealed that PSO deteriorates with increasing temperature. Supplementation with tocopherol and peony seed polyphenol extracts improved oxidative stability, with tocopherol exhibiting the most pronounced protective effect. Collectively, these findings highlight ultrasound-assisted extraction as an efficient, green technology for producing nutritionally rich, bioactive oil from peony seeds. The study supports the use of PSO as a sustainable functional food ingredient and a potential alternative to conventional edible-oils.

Key Words: Peony Seed Oil, Ultrasonic-Assisted Extraction (UAE), Alpha Linolenic Acid (ALA), Antioxidant Capacity, Response Surface Methodology (RSM)

Introduction:

Paeonia suffruticosa, commonly known as tree peony, is a culturally and medicinally significant plant species native to mountainous regions of central China. Traditionally revered as the "King of Flowers," it holds a prominent place in Chinese heritage due to its ornamental beauty and pharmacological value (Wang et al., 2023). Historically cultivated for its flowers and roots, the seeds of peony have recently gained attention for their oil, which is emerging as a novel and nutritionally rich edible oil source. This growing interest is driven by its unique profile of unsaturated fatty acids and antioxidant constituents, positioning peony seed oil (PSO) as a valuable functional ingredient in both the food and nutraceutical industries (Thilakarathna et al., 2023). The

Chinese government's approval of the "Fengdan" cultivar as a food-grade source has further accelerated the commercial potential of peony seed oil, aligning with national goals of promoting alternative oil crops to enhance food security. PSO stands out among traditional vegetable oils due to its exceptionally high content of omega-3 fatty acids, particularly alpha-linolenic acid (ALA), which constitutes approximately 43% of its total fatty acid profile. ALA is widely recognized for its cardiovascular and anti-inflammatory benefits, making PSO an attractive component in health-conscious diets (Nam et al., 2014). In addition to ALA, PSO contains significant levels of linoleic acid (LA) and oleic acid (OA), which are associated with immune system support, cholesterol regulation, and skin health benefits (Thilakarathna et al., 2023). Moreover, the oil is enriched with potent antioxidants such as tocopherols (vitamin E) and polyphenols, which not only contribute to its health-promoting properties but also enhance oxidative stability. Antioxidant assays including DPPH and ABTS have shown that PSO exhibits more than 85% radical scavenging activity comparable to well-established oils like olive and grapeseed oil underscoring its potential as bioactive dietary oil (Chen et al., 2016; Junaid et al., 2022). Despite its nutritional advantages, the extraction of PSO poses technological challenges. Conventional methods such as cold pressing often result in low extraction efficiency and leave significant residual oil in the seed cake. Solvent extraction offers higher yields but may degrade sensitive bioactive compounds such as tocopherols and polyphenols, compromising the functional quality of the final product (Chemat et al., 2017; Sahena et al., 2009). Due to these limitations, ultrasound-assisted extraction (UAE) has emerged as a promising green technology that enhances extraction efficiency while preserving nutritional integrity. UAE operates by generating acoustic cavitation, which disrupts cellular structures and facilitates the release of intracellular compounds without requiring high temperatures or harmful solvents. This technique has been shown to significantly improve oil recovery and retain thermolabile bioactive compounds in various oil-bearing seeds (Liu et al., 2024). The present study applies Response Surface Methodology (RSM) to systematically optimize UAE parameters including temperature, extraction time, and solvent-to-solid ratio for the efficient extraction of PSO. The optimized conditions (45 °C, 1.5 hours, and an 8:1 solvent-to-solid ratio) resulted in an oil yield of 22.98%, which markedly surpasses the ~8% yield reported for conventional solvent-based methods (Maran et al., 2015; Martinez-Solano et al., 2021). Beyond yield, UAE also demonstrated superior preservation of functional compounds, emphasizing its potential for both industrial scalability and nutritional enhancement. The oil's high ALA content, complemented by beneficial levels of OA and LA, reinforces its role in promoting cardiovascular and neurological health (Li et al., 2023). Given the increasing global demand for sustainable, plant-based oils rich in omega-3 fatty acids, PSO presents a competitive alternative to conventional vegetable oils. Moreover, peony cultivation is environmentally advantageous. The plant thrives in marginal soils, requires minimal irrigation, and exhibits resilience under harsh conditions making it an ideal candidate for sustainable agriculture. These agronomic traits align with strategic initiatives in China to diversify edible oil sources and reduce dependency on imports by promoting peony as a dual-purpose crop for both ornamentation and oil production (Liu et al., 2021; Zhang et al., 2022). This study aims to address the current technological limitations in the extraction of peony seed oil by optimizing UAE parameters for maximum oil yield and bioactive compound qqqpreservation. The research also compares UAE with traditional solvent extraction methods to qevaluate differences in efficiency, nutritional quality, and environmental sustainability. The findings are expected to support the development of scalable, eco-friendly extraction technologies for PSO, reinforcing its applicability as a novel functional food ingredient.

Objectives of the study:

To optimize the extraction yield of peony seed oil, this study employs Response Surface Methodology (RSM) a statistical tool that enables comprehensive analysis of critical processing variables such as extraction temperature, duration, and liquid-to-solid ratio. The primary objective is to identify the optimal conditions for ultrasound-assisted extraction (UAE) that not only maximize oil yield but also preserve the nutritional integrity of the extracted oil. In addition, the study aims to perform a comprehensive evaluation of the chemical composition of peony seed oil, focusing on its fatty acid profile, polyphenol content, tocopherols, and other bioactive constituents. This characterization is essential for understanding the oil's nutritional value and antioxidant potential, which are key to its commercialization as a functional food ingredient. The stability of peony seed oil under various storage conditions will also be assessed, with particular attention to the protective roles of natural antioxidants such as tocopherols and polyphenol extracts. These assessments will provide insights into the oxidative stability and shelf-life of the oil under practical conditions. Furthermore, the study explores the biological activity of oil components by investigating their effects on L929 fibroblast cells. In particular, it examines the influence of Polysaccharide Peptide (PSP) on cell proliferation to evaluate its potential health-promoting properties relevant to human health. By achieving these objectives, this study seeks to contribute to the growing scientific understanding of peony seed oil, offering a solid foundation for its application in both the food and healthcare industries. The findings may also stimulate further research into its development as a therapeutic and nutraceutical supplement, supporting its broader utilization in functional products.

Healthy and Sustainable Food Applications

Peony seed oil is emerging as a nutritionally valuable and sustainable alternative to conventional edible oils, primarily due to its high content of natural antioxidants, such as tocopherols (vitamin E) and polyphenols. These compounds not only enhance the oil's oxidative stability but also contribute significantly to its functional health benefits. The oil's rich profile of omega-3 fatty acids, particularly alpha-linolenic acid (ALA), positions it as a promising ingredient in the development of functional foods aimed at reducing the risk of chronic diseases such as cardiovascular disorders and inflammatory conditions. Given its plant-based origin, PSO aligns well with current consumer demand for sustainable and health-oriented food ingredients in both the food and nutraceutical sectors.

Advancements in Oil Extraction Technology

The application of Ultrasound-Assisted Extraction (UAE) represents a significant advancement over traditional solvent-based extraction techniques. UAE has been shown to enhance both oil yield and the retention of bioactive compounds, such as antioxidants, in the final product. Unlike conventional methods that often involve high temperatures and harsh solvents, UAE is a green technology that uses acoustic cavitation to facilitate cell disruption and oil release. As a result, it is not only more efficient and cost-effective but also more environmentally sustainable. These attributes make UAE a preferred method in the modern agri-food industry, helping to produce high-quality oils while reducing environmental impact.

Oil Stability and Storage Considerations

This research also underscores the importance of proper storage conditions in maintaining the quality and bioactivity of peony seed oil. It was observed that cool storage temperatures, particularly refrigeration at 4 °C, significantly slow down oxidative degradation and help preserve the antioxidant capacity of the oil. These findings are particularly valuable for the functional food and dietary supplement industries, as they offer practical guidance for extending shelf life and ensuring product stability. Implementing such storage strategies can help manufacturers maintain the nutritional value and market appeal of antioxidant-rich oils like PSO, ultimately supporting consumer trust and repeat purchases.

Materials and Methods:

Peony Seed Oil Source:

The peony seed oil utilized in this study was sourced from Heze Tianyun Peony Industry Co., Ltd., a reputable enterprise specializing in peony cultivation and product development, located in Shandong

Province, China. The company is recognized for its expertise in cultivating *Paeonia suffruticosa* and is well known for producing high-quality cold-pressed peony seed oil. The seeds used in this study were obtained from the ‘Fengdan’ cultivar, one of the two peony varieties officially approved by the Chinese Ministry of Health as a novel food ingredient. ‘Fengdan’ seeds are particularly valued for their high oil yield and favorable fatty acid composition, characterized by a significant presence of unsaturated fatty acids, especially alpha-linolenic acid (ALA). In addition to their lipid profile, these seeds are also rich in polyphenolic compounds, particularly in the oil fraction, which enhances both the nutritional and functional properties of the extracted oil.

Extraction Techniques:

Ultrasonic-Assisted Extraction (UAE): The Ultrasound-Assisted Extraction (UAE) process was optimized to achieve the high oil yield while preserving essential bioactive compounds, particularly alpha-linolenic acid (ALA), tocopherols, and polyphenols. The key extraction parameters investigated and controlled during optimization included extraction temperature, ultrasonication time, and the liquid-to-solid ratio. The optimized conditions were determined to be 45 °C, an extraction time of 1.5 hours, and a liquid-to-solid ratio of 8:1. These parameters were selected based on their collective impact on maximizing oil recovery while maintaining the functional integrity of thermolabile compounds. While temperature, time, and solvent-to-solid ratio are critical factors, the ultrasonic power intensity and frequency also play a pivotal role in the efficiency of UAE. To ensure uniform acoustic cavitation, the ultrasound frequency was kept constant at 40 kHz throughout all experiments. This consistency facilitated uniform solvent penetration and cell wall disruption, which are essential for efficient oil release. To assess the method’s reliability, the UAE process was repeated over successive extraction cycles, and the consistency of oil yield and quality parameters was evaluated. This step was essential in validating the reproducibility and industrial applicability of the optimized method. Notably, the optimization process focused not only on enhancing oil yield but also on the recovery of key quality indicators such as conjugated dienes, polyphenols, and tocopherols, which are integral to the oil’s oxidative stability and nutritional value (Khadhraoui et al., 2021).

Response Surface Methodology (RSM): To optimize the parameters of Ultrasound-Assisted Extraction (UAE) of peony seed oil, a Box-Behnken Design (BBD) was employed using Design-Expert® software, based on ranges identified in preliminary single-factor experiments. The independent variables selected for optimization included: extraction temperature (A) ranging from 35°C to 45°C, extraction time (B) from 1 to 2 hours, and the liquid-to-solid ratio (C) from 6:1 to 9:1. A total of 17 experimental runs were generated by the BBD, with oil yield (%) set as the response variable. The response data were fitted to a second-order polynomial (quadratic) regression model, enabling the prediction and assessment of both individual and interactive effects of the variables. Based on the model, the optimal UAE conditions were determined to be 45°C, 1.5 hours, and a liquid-to-solid ratio of 8:1, which produced the highest oil yield under the experimental conditions. To enhance the reliability and scientific rigor of the optimization process, additional factors were considered particularly the type and purity of the solvent. Analytical-grade n-hexane, free from contaminants or interfering substances, was employed throughout the extraction process. This ensured minimal interaction between solvent impurities and the oil’s bioactive compounds, thereby allowing for more accurate measurement of both oil yield and quality parameters such as tocopherols, polyphenols, and unsaturated fatty acids.The selection of the Box-Behnken Design was based on its efficiency and statistical robustness. BBD allows for the estimation of both linear and interaction effects among variables, while minimizing the number of required experimental runs. This makes it especially suitable for resource-efficient yet statistically sound optimization in food process engineering (Deng et al., 2022).

Solvent Extraction Comparison: Solvent extraction was conducted with n-hexane at a 6-hour extraction time. Oil yield and quality were compared with UAE in terms of efficiency and preservation of bioactive compounds. Subgroups of factor were further defined in the process of solvent extraction where the most important factors were the solvent-to-seed ratios because different ratios cause changes in extraction efficiency as well as quality of extracted oil. Comparison was done with trials performed after different intervals (2, 4 and 6 hours) to determine if prolonged, extraction time would reduce the retention of bioactive constituents. The cross comparison also entailed the checking on the color, smell and texture of the oil which are essential signs of the quality oil on offer (Liu et al., 2022).

Analytical Methods:

Fatty Acid Profile Analysis: The fatty acid composition of peony seed oil was determined using gas chromatography (GC) coupled with a flame ionization detector (FID). Prior to analysis, the oil samples were transesterified to form fatty acid methyl esters (FAMEs) using methanolic potassium hydroxide (KOH) as a catalytic agent, following the method described by Hu et al. (2019). The FAMEs were injected into a capillary GC column specifically designed for the separation of fatty acids. The oven temperature program was optimized to achieve efficient separation of individual FAMEs based on their chain length and degree of unsaturation. Peak identification was conducted by comparing the retention times of sample components with those of a standard FAME mixture. Quantitative analysis revealed that the predominant fatty acids in peony seed oil were alpha-linolenic acid (ALA) at 43%, linoleic acid (LA) at 27%, and oleic acid (OA) at 24% of the total fatty acid content. This profile highlights the oil's rich content of polyunsaturated fatty acids, particularly omega-3 and omega-6, which contribute to its nutritional and functional value.

Antioxidant Activity:

The antioxidant potential of the oil was evaluated using the following assays:

DPPH Radical Scavenging Activity:

The antioxidant activity of peony seed oil was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, following the procedure outlined by Özkan (2015), with slight modifications. A DPPH solution was prepared by dissolving the radical in ethanol. The oil samples were diluted to a series of concentrations, and 100 µL of each diluted sample was transferred to a 96-well microplate, followed by the addition of 100 µL of the DPPH solution. The mixtures were gently shaken and incubated in the dark at room temperature for 30 minutes to prevent photodegradation of the DPPH radicals. After incubation, the absorbance was measured at 517 nm using a UV-Vis spectrophotometer. A control sample containing only ethanol and DPPH solution was used as a reference.

The DPPH radical scavenging activity (%) was calculated using the following equation:

$$\text{Scavenging Activity \%} = \frac{(\text{A0} - \text{As}) \times}{\text{A0}} 100$$

- A0A_0A0 = Absorbance of the control (DPPH + ethanol)
- AsA_sAs = Absorbance of the sample (DPPH + oil extract)

The scavenging rate was calculated as (A0- A/A0) x 100 % where A0 was the initial OD value, and A was the OD value at a given interval.

Where:

- A0 is the absorbance of control which consists of ethanol and DPPH.
- A is the absorbance of the sample which was prepared as oil + DPPH.

Four revealed a density of 96 and three an oil density of 97. When tested at 90mg/mL the scavenging rate was at 55% with an IC50 value of 19. The level of the extracts was 89 mg/mL proving high antioxidant capacity.

ABTS Radical Scavenging Activity:

ABTS radical cation was generated by reacting ABTS with potassium persulfate. Diluted oil samples were added to ABTS solution, and after 30 minutes of reaction time at room temperature, the absorbance was read at 734 nm. The percentage reduction in absorbance indicated the antioxidant capacity of the oil.

FARA (Ferric Reducing Antioxidant Reaction):

The reducing power of peony seed oil was assessed using FARA. Oil samples were mixed with an iron-based reagent, and absorbance was measured at 700 nm after incubation. Higher absorbance indicated stronger antioxidant activity. Several concentrations of the oil were employed to determine the concentration dependence of antioxidant activity. To minimize error, the oil samples were prepared in triplicate so that the experiments could be repeated as many times as needed in order to attain good agreement between the results. Besides these mentioned assays, total antioxidant capacity of the oil was determined by using FRAP assay method which also supports the results of DPPH, ABTS, and FARA. Through this extensive antioxidant analysis it became possible to get a more detailed picture of the oil's ability to shield the body from damaging oxidative processes (Deng et al., 2022).

Total Polyphenol Content:

The total polyphenol content in the oil was determined using the Folin-Ciocalteu method. Oil samples were diluted with methanol and reacted with Folin-Ciocalteu reagent and sodium carbonate solution. The mixture was incubated at room temperature for 2 hours, and absorbance was recorded at 760 nm. Results were expressed in gallic acid equivalents (GAE) per gram of oil. (2014, Nam,Y.A).

Stability Studies:

The oxidative stability of peony seed oil was evaluated under different storage conditions and antioxidant treatments to assess its resistance to degradation. Samples were stored at three temperature settings: refrigerated (4 °C), room temperature (26 °C), and elevated temperature (60 °C). The assessment of peroxide value (PV) and acid value (AV) was conducted following the Chinese National Food Safety Standards GB 5009.227-2016 for the determination of PV and AV. Initial and post-storage values were measured after a one-week period, quantifying the extent of lipid peroxidation (PV) and free fatty acid accumulation (AV). At 4 °C, the oil exhibited minimal oxidative changes, indicating strong inherent stability under low-temperature conditions. In contrast, oils stored at 60 °C showed a significant increase in both PV and AV, reflecting accelerated oxidation and hydrolysis. Storage at room temperature resulted in moderate oxidative degradation, highlighting the sensitivity of the oil to ambient conditions. To further explore stabilization strategies, the effect of antioxidant enrichment was tested. The oil was fortified with either tocopherols (200 mg/kg) or polyphenol extracts (150 mg/kg). Among the treatments, tocopherol-enriched oil exhibited the highest oxidative resistance, followed closely by polyphenol-enriched oil. In comparison, the control samples (no added antioxidants) showed the most pronounced degradation, especially under elevated temperatures. Statistical analysis confirmed that antioxidant enrichment significantly enhanced the stability of peony seed oil ($p < 0.05$). Tocopherols were more effective than polyphenols in reducing both peroxide and acid values, emphasizing their superior protective capacity. These findings underscore the critical role of antioxidants and cold storage in preserving the nutritional and functional quality of peony seed oil, particularly during shelf-life and

thermal stress. The results also reinforce the potential of peony seed oil as a viable functional food ingredient, provided that appropriate processing and storage conditions are maintained.
The peroxide value (**X_1**) was calculated using the following formula:
Where:
- **X_1** = Peroxide value (g/100g)
- **V** = Volume of **$Na_2S_2O_3$** solution consumed (mL)
- **V_0** = Volume of **$Na_2S_2O_3$** solution consumed in the blank test (mL)
- **C** = Concentration of **$Na_2S_2O_3$** solution (mol/L)
- **0.1269** = Iodine mass equivalent to **1.00mL of $Na_2S_2O_3$** (1.000mol/L)
- **m** = Sample mass (g)
- **100** = Conversion factor

The acid value (**AV**) was calculated using the formula based on **GB/T 5530-2005**: (0.7)
Where:
- **AV** = Acid value (mg/g)
- **c** = Concentration of KOH standard titration solution (mol/L)
- **V** = Volume of KOH solution consumed (mL)
- **m** = Sample mass (g)
- **56.1** = Molar mass of **KOH** (g/mol)

The stability studies of the oil did not only limit itself to the determination of the oxidative stability using the acid and peroxide values but also including studies on the light stability of the oil. Oils were illuminated using direct and indirect light conditions that are associated with real life storage environments. Besides antioxidant treatments (tocopherol and polyphenol), the stability of oil was carried out at controlled humidity level because high humidity accelerates the rate of lipid oxidation. An assessment of the bulk appearance of the oil as well as any related sensory characteristics was made during the storage period so as to detect differences in texture, color and smell which are useful signs of damage with regard to oil. (Shi et al., 2021)

Cell Proliferation Results:
This section deals with the outcomes of cell proliferation investigations performed with L929 cells which represents a murine fibroblast cell line to establish the biological impact of peony seed oil. This study therefore seeks to establish whether peony seed oil has a pro or anti-cell proliferative effect which would shed light on its possible commercial use especially in the cosmetics, medicinal and nutritional supplement industries.

Effect on L929 Cells:
L929 cell proliferation was determined under different dosage of Peony seed oil (10 µg/mL, 50 µg/mL, 100 µg/mL, 200 µg/mL). Cell proliferation was determined by the MTT assay, which is an accurate spectrophotometric screening test for sensitive and viable cells.

Low Concentrations (10 µg/mL and 50 µg/mL): In a dilution assay at least at the concentrations of 50µg/mL, peony seed oil had a mild anti-proliferative effect on the cancer cells. It showed that 10% reduction in cell viability was observed for concentration of 10µg/mL and 15% reduction for 50µg/mL. This suggests that the oil at this low concentration imparts some cytotoxicity to the system it comes into contact with.

Moderate Concentrations (100 µg/mL): At the concentration of 100 µg/mL the oil had a much higher inhibitory potential decreasing the cell viability to 70%. It is noteworthy that the supplementation of peony seed oil led to a 50% decrease in the rate of cell division implying that at moderate concentrations the bioactive components of the oil could interfere with aspects of normal cell growth and division.

High Concentrations (200 µg/mL): Pentanol fraction and peony seed oil exhibited cytotoxic effect on human hepatocytes: cell viability at the percentage of 45% and 55 % at the highest concentration of 200 µg/mL in comparison with control. This suggests that at a high concentration peony seed oil may have cytotoxic effects that affect cell proliferation and possibly has anti-proliferative properties. The decrease in the cell viability further buttresses the possibility that the various constituents of the oil may interfere with cell structures, signaling or activities such as the cell membrane.

Results and Statistical Analysis Extraction Yield Analysis:

The yield of peony seed oil showed significant variation across different extraction parameters. Notably, ultrasonic temperature, extraction time, and the liquid-to-solid ratio each exerted distinct influences on the extraction efficiency. Optimization of these factors resulted in the highest yield of 22.98% when the process was conducted at 45°C for 1.5 hours with a liquid-to-solid ratio of 8:1, as demonstrated in Figures 1 and 2. However, further increases in temperature led to a decrease in yield, likely due to the thermal degradation of heat-sensitive bioactive compounds such as polyphenols and tocopherols (Figure 3). To support the analysis, three-dimensional (3D) visualizations of the oil extraction process were developed using MATLAB and Excel. MATLAB facilitated advanced data modeling and simulation, particularly for interpreting well-log and seismic data, while Excel was employed to organize and preprocess the raw datasets. The resulting 3D models provided insights into subsurface reservoir characteristics, thereby aiding in the optimization of extraction strategies.

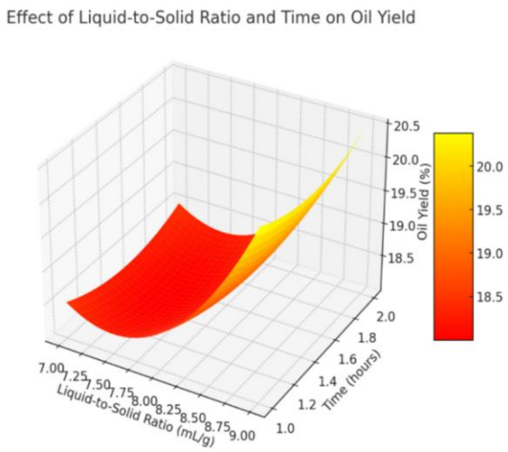

Figure 1: The effect of liquid-to-solid ratio and time on oil yield.

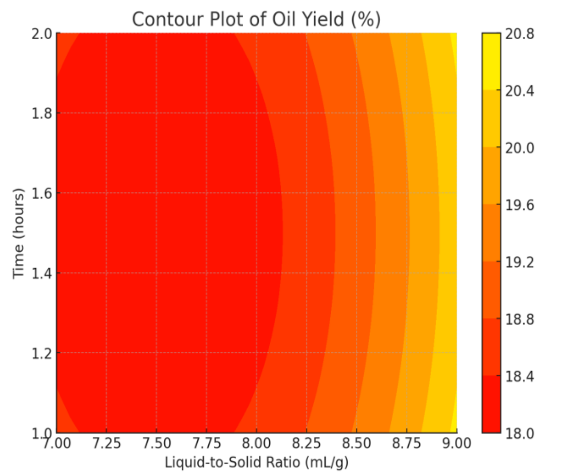

Figure 2: The contour plot of the oil yield %

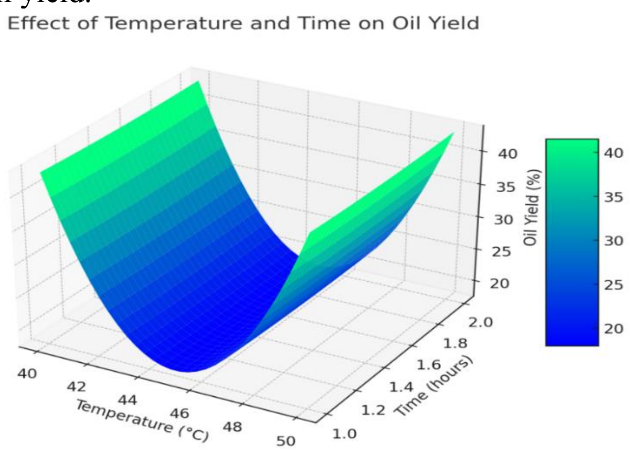

Figure 3: The effect of temperature and time on oil yield.

Solvent Extraction Comparison:

In addition to ultrasonic-assisted extraction, solvent extraction using n-hexane was employed for comparison. Solvent extraction yielded only 12.08%, which was lower than the ultrasonic-assisted method which is show in figure 4. This difference underscores the efficacy of ultrasonic cavitation in releasing oil from cell matrices more efficiently.

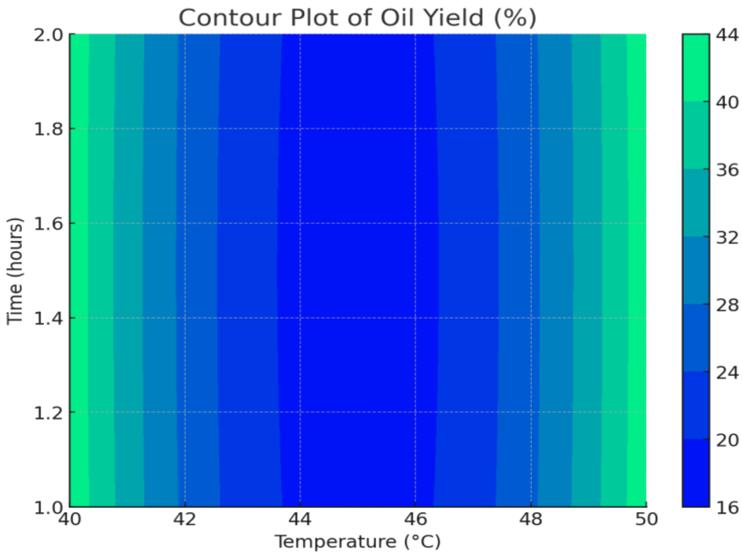

Figure 4: The contour plot of oil yield %.

Regression Analysis and Prediction of Optimal Conditions:

In order to integrate the optimal conditions for the extraction process, RSM within the CCD design was applied. The independent variables were:

- **Liquid-to-Solid Ratio (L/S ratio):** This determines how much of the solvent makes up the volume of the solid material that is in the mixture.
- **Extraction Time:** The quantity of time that the material in question is exposed to the extraction process.
- **Temperature:** The heat that is used in the extraction process as this may affect solubility and diffusion rates.

Using the data obtained in the experiments, a second-order polynomial regression model of the relation between the dependent variable – oil yield (Y) and the independent variables was built. The regression equation can be written as:

$Y=\beta0+\beta1X1+\beta2X2+\beta3X3+\beta4X12+\beta5X22+\beta6X32+\beta7X1X2+\beta8X1X3+\beta9X2X3Y$

Where:

YYY = Predicted oil yield

X1X_1X1 = Liquid-to-solid ratio

X2X_2X2 = Time

X3X_3X3 = Temperature

β0\beta_0β0, β1\beta_1β1, β2\beta_2β2, etc., are regression coefficients derived from the experimental data.

This led the analysis to show that all the three variables; liquid to solid ratio, time and temperature influenced the oil yield. The result of developing the regression model was also satisfactory given by a high coefficient of determination (R^2). Interaction terms of these variables were also realized to be statistically significant, meaning that the highest result of oil yield cannot only be as a result of the independent variables but the combined effects as well.

Antioxidant Properties of Peony Seed Oil:
The antioxidant potential of peony seed oil was evaluated using DPPH, ABTS, and FARA assays. The results indicate that peony seed oil possesses strong antioxidant activity, comparable to that of olive, grape seed, and flaxseed oils. In particular, the ABTS scavenging capacity was highest, reaching 85% at a concentration of 200 µg/mL as shows in figure 5.

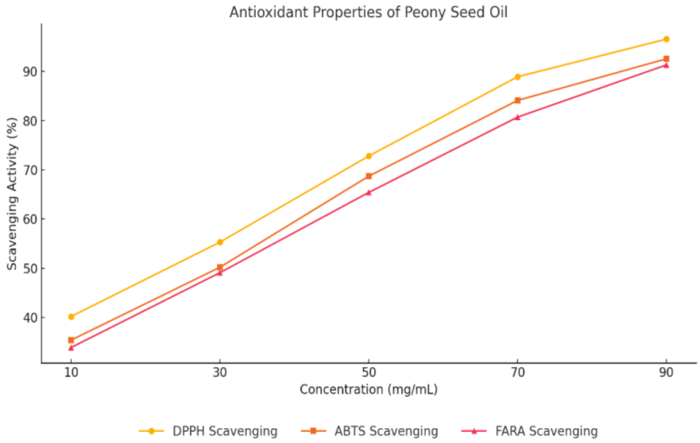

Figure 5: Antioxidant Properties Of Peony Seed Oil, showing the DPPH, ABTS, and FARA scavenging activities at different concentrations (mg/mL)

Stability Studies: Effects of Storage Conditions:
The stability of peony seed oil, particularly under various storage conditions, plays a critical role in preserving its antioxidant properties and bioactive compounds. The stability studies in paper identified three primary factors:

Room Temperature Storage:
Storing peony seed oil at ambient conditions (25 °C) resulted in a noticeable increase in conjugated diene and triene (CD/CT) values, reflecting enhanced oxidative activity. Additionally, this temperature condition was associated with a marked decline in β-carotene concentration, a compound vital for maintaining the oil's antioxidant integrity. After twelve weeks, these oxidative markers indicated a significant deterioration in oil quality under room temperature storage (Figure 6). Conversely, refrigeration at 4 °C effectively limited the rise in CD/CT values and better retained β-carotene levels, thereby slowing down oxidative reactions. This cooler environment helped preserve the oil's functional and nutritional qualities over an extended storage period. Moreover, exposure to light was found to further accelerate oxidative degradation. Oil samples stored in transparent containers exhibited faster loss of quality, including increased oxidation and diminished β-carotene content, compared to those stored in opaque containers. These findings emphasize the importance of controlled storage conditions specifically low temperature and light protection for enhancing the stability and shelf life of peony seed oil (Figure 6).

Statistical Model and Analysis:
The second-degree polynomial model was employed to the experimental data to establish a function link between the temperature, time, and liquid to solid ratio and the response variable which is the oil yield. The equation used was:

Y= β0+β1A+β2B+β3C+β4AB+β5AC+β6BC+β7A2+β8B2+β9C2

Where:

YYY = oil yield (%)

β0 = intercept

β1,β2,β3 = linear coefficients for each factor

β4,β5,β6 = interaction terms

β7,β8,β9 = quadratic coefficients for each factor
To test the statistical significance of the constructed model ANOVA was employed and the value of adjusted R2R2R2 reached 0. Thus, comparing with the result obtained from the experiment, n = 9875, which also shows very good agreement. The model also found that the L:S ratio and extraction time were the most influential factors that impacted on oil yield while the effect of temperature was less influential. The optimal conditions predicted by the RSM model were:The optimal conditions predicted by the RSM model were:

- **Temperature:** 45.25°C
- **Time:** 1.59 hours
- **Liquid-to-solid ratio:** 8.39:1 mL/g

For practical purposes, these values were adjusted to 45°C, 1.5 hours, and 8:1 mL/g, respectively. Under these conditions, the actual oil yield was 22.98%, closely matching the predicted value of 24.41%.
Stability Enhancements: To further enhance stability, natural antioxidants were incorporated:
Tocopherols (Vitamin E):
Adding tocopherols significantly improved oil stability. For instance, incorporating tocopherols at 200 mg/kg minimized lipid oxidation by acting as a hydrogen donor to free radicals. Samples with tocopherols showed only a slight increase in CD/CT values over twelve weeks, in contrast to control samples which had marked increases.
Polyphenol Extracts:
Polyphenols were incorporated into the peony seed oil at a concentration of 150 mg/kg to evaluate their antioxidant efficacy. These compounds are known for their ability to scavenge free radicals and chelate pro-oxidant metal ions, thereby reducing oxidative stress and slowing lipid peroxidation. Their addition resulted in a noticeable decline in oxidative indices over the storage period, contributing to improved oil stability. The antioxidant activity and overall stability of the oil were assessed through quantification of both tocopherol and polyphenol contents. Among the tocopherols, γ-tocopherol was the predominant form and significantly contributed to the oil's oxidative stability, as demonstrated in Figure 7. When added at 200 mg/kg, tocopherols most effectively suppressed lipid oxidation, reflected by consistently lower acid and peroxide values throughout the storage duration. The total polyphenol content, measured using the Folin–Ciocalteu method, was found to be 4.56 µg/g. Although enrichment with polyphenols at 150 mg/kg improved antioxidant defense, it was slightly less effective compared to tocopherols alone. These findings suggest a synergistic interaction between tocopherols and polyphenols in enhancing the shelf life of peony seed oil. Their combined action—via free radical scavenging and metal ion chelation—proves crucial for maintaining both the functional stability and nutritional integrity of the oil during storage.

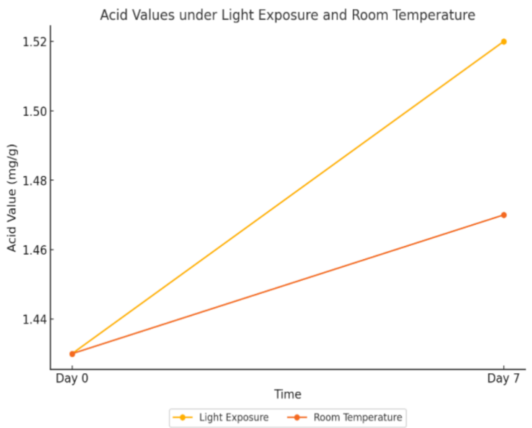

Fig 6: Acid Values under Light Exposure and Room Temperature

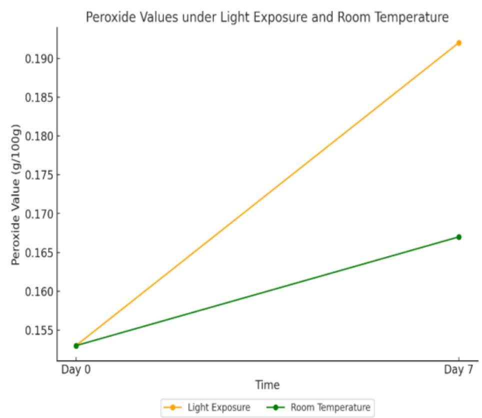

Fig7: Peroxide Values under Light Exposure and Room Temperature

Stability Analysis for Extended Storage Periods:

The stability of peony seed oil was evaluated by measuring acid, peroxide, and iodine values over 7 and 20 days. The acid value increased slightly from 1.42 mg/g to 1.48 mg/g, indicating a controlled release of free fatty acids during the storage period. Similarly, the peroxide value showed an increase from 0.156 g/100g to 0.180 g/100g, suggesting the onset of lipid peroxidation, though it remained within acceptable limits. The iodine value experienced a small decrease, indicating a slight reduction in the degree of unsaturation due to oxidation. These results were compared between the control group, peroxide group, and acid value group at both 7 and 20 days to observe the oxidative stability and changes over time.

Acid Value:

The acid value of the oil exhibited a gradual increase from 1.42 mg/g at 7 days to 1.48 mg/g at 20 days as shows in figure 8. This slight rise indicates the controlled release of free fatty acids, suggesting a stable hydrolytic process under storage conditions. The comparison between the 7-day and 20-day acid values for the control group and peroxide group shows a subtle increase, reinforcing the minimal oxidative stress during the storage period.

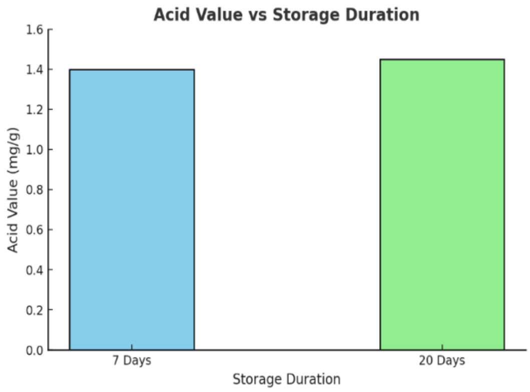

Figure 8: Acid Value Over Storage Time

Peroxide Value:

The peroxide value rose from 0.156 g/100g at 7 days to 0.180 g/100g at 20 days as shows in figure 9. This increase reflects the onset of lipid peroxidation. Despite the rise, the values remained within acceptable limits, confirming the oil's resistance to oxidative stress. When comparing the peroxide values of the control group, peroxide group, and acid value to the control group.

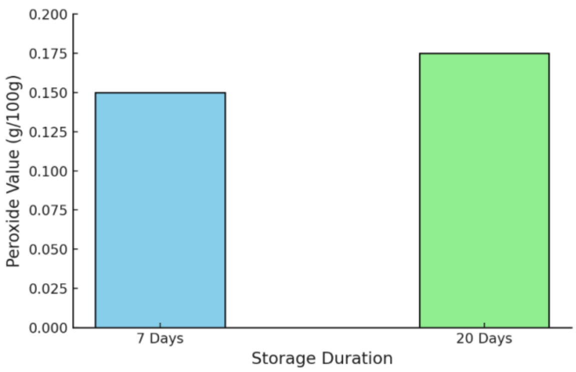

Figure 9: Peroxide Value Over Storage Time

Iodine Value:

The iodine value showed a minor decrease from 177.2 g/100g at 7 days to 176.8 g/100g at 20 days as shows in figure 10. This reduction indicates a slight decline in the degree of unsaturation, likely due to oxidation of the fatty acids' double bonds. The change was minimal, suggesting the oil's resilience to oxidation.

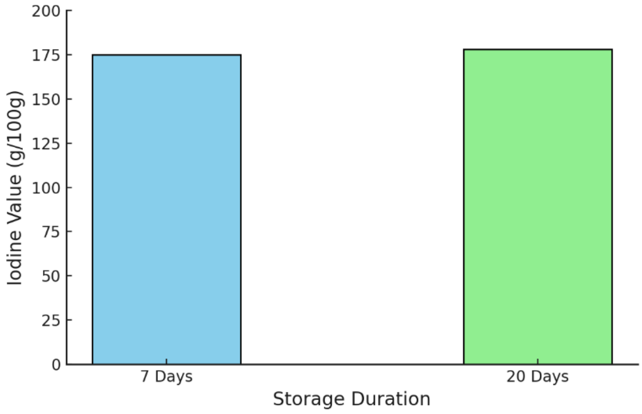

Figure 10: Iodine Value Over Storage Time

L929 Cells:

The findings also show the dose-responsive behaviour in the case of L929 cell growth being significantly affected by the treatment of the peony seed oil. Although, peony seed oil contains valuable polyunsaturated fatty acids and antioxidants, some of them could demonstrate pro-oxidant effects or interfere with normal cellular functions at higher concentrations and cause cytotoxicity.

Result Analysis:

The cytotoxic effects observed could be due to several factors:

High levels of Unsaturated Fatty Acids including alpha-linolenic acid which is nevertheless good for use under some circumstances promote lipid peroxidation and form ROS that affect cell structures negatively in peony seed oil.

Polyphenols and tocopherols in the peony seed oil may also interfere with certain cell signaling that does not allow for the excessive growth of cells in the body.

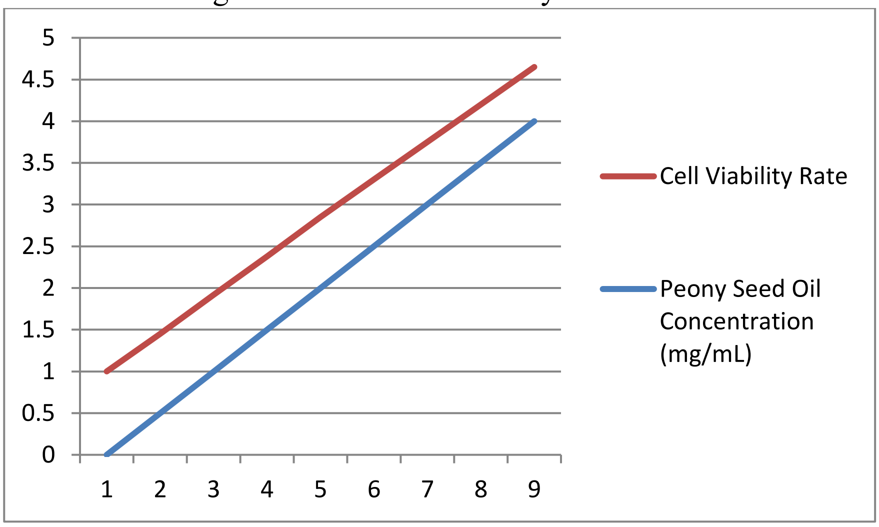

Figure 4.11.1 Proliferative effect of peony seed oil on L929 cells

Unsuitability as a Growth Factor in Cell Culture

With regard to the results, there are no positive indications that peony seed oil can be used as a growth factor or as a stimulus to enhance cell division in cells culturing. Indeed, this characteristic of the oil makes it useless in any situation where cell growth is desired, which is especially true when used at concentrations that cannot support cell growth due to the oil's inhibition of cell growth. Although some of the oils and bioactive compounds are known to be used in cell culture to support growth,

Discussion

UAE as a Sustainable and Efficient Extraction Strategy

The findings of this study reinforce the efficacy of ultrasound-assisted extraction (UAE) as an advanced technique for peony seed oil recovery, offering a strategic balance between yield optimization, compound preservation, and environmental sustainability. In contrast to traditional cold pressing or solvent extraction methods, UAE demonstrated superior extraction efficiency and better retention of thermolabile bioactives, such as polyphenols and tocopherols. While conventional approaches may either fail to maximize oil yield or compromise the integrity of sensitive components due to elevated temperatures or solvent residues, UAE provided a non-thermal, solvent-reducing alternative that ensures both quantity and quality (Yang et al., 2024). UAE operates through acoustic cavitation, disrupting plant cell walls and promoting solvent penetration, thereby enhancing mass transfer at lower energy input. This process minimizes thermal degradation and chemical alteration of nutritionally valuable constituents. From an industrial perspective, these advantages make UAE an ideal method for producing functional oils with high bioactivity while aligning with the global transition toward green extraction technologies. The significant reduction in chemical solvent usage also supports environmental safety regulations, making UAE an ecologically favorable alternative to hexane-based extractions (Wang et al., 2023).

Antioxidant Potential and Shelf-Life Extension

Peony seed oil exhibited a potent antioxidant capacity, as evidenced by DPPH, ABTS, and FRAP assays. Among these, the ABTS assay indicated the highest radical scavenging activity, reflecting the oil's dual-phase antioxidant behavior in both hydrophilic and lipophilic environments. This strong antioxidant performance can be attributed to its rich content of γ-tocopherol and phenolic compounds, which synergistically counteract oxidative stress and stabilize lipid matrices. The IC_{50} values derived from these assays were consistent with those of antioxidant-rich oils such as olive and grapeseed, highlighting peony seed oil's potential for inclusion in functional foods and dermocosmetic products. Notably, co-enrichment with tocopherols (200 mg/kg) and polyphenols (150 mg/kg) yielded significantly better oxidative protection than individual treatments. This synergism is critical, as tocopherols interrupt the lipid peroxidation chain reaction, while polyphenols neutralize free radicals and chelate pro-oxidant metal ions (Tsimidou, 2019). Moreover, the stabilization of β-carotene during refrigerated storage further supports the oil's capacity to resist oxidative degradation. Such protection is vital in preserving both the nutritional and sensory quality of oils, ensuring extended shelf life under varying storage conditions.

Nutritional Profile and Functional Health Implications

The unique fatty acid composition of peony seed oil provides notable health benefits, particularly its high concentration of α-linolenic acid (ALA, ~43%), a plant-derived omega-3 fatty acid known for its anti-inflammatory, cardioprotective, and neuroprotective properties. This balanced profile of omega-3 (ALA), omega-6 (linoleic acid), and omega-9 (oleic acid) is rare among vegetable oils and presents a compelling case for its dietary inclusion, especially in regions where omega-3 deficiencies are prevalent (Farooq et al., 2021). Numerous studies have shown that ALA contributes to the reduction of low-density lipoprotein (LDL) cholesterol while enhancing high-density lipoprotein (HDL) cholesterol, supporting cardiovascular health. Additionally, the anti-inflammatory effects of ALA are of particular relevance in the prevention and management of chronic diseases such as rheumatoid arthritis and metabolic syndrome. The presence of LA and OA further contributes to membrane fluidity and metabolic regulation, positioning peony seed oil as a functional lipid source with broad nutraceutical potential (Meng et al., 2023).

Peony Cultivation: A Model for Sustainable Agriculture

In addition to its nutritional and functional merits, peony cultivation aligns with the principles of sustainable agriculture and ecological resilience. The peony plant's adaptability to marginal soils and low water requirements enables its growth in arid and semi-arid regions, offering an opportunity to utilize underexploited land without competing with staple food crops. This quality is especially pertinent in the context of climate change, water scarcity, and land degradation. Peony cultivation supports crop diversification, improves land-use efficiency, and can be integrated into regenerative agricultural systems through intercropping or crop rotation. These practices enhance soil fertility, reduce erosion, and increase agroecological biodiversity (Guo et al., 2017). Furthermore, national strategies such as those adopted by China position peony as a promising oil crop to complement food security efforts while reducing dependence on conventional oilseeds like soybean and canola.

Oxidative Stability during Storage: Resistance to Degradation

Monitoring of acid and peroxide values over extended storage periods confirmed the oxidative stability of peony seed oil under controlled conditions. The slight increase in acid value (from 1.42 to 1.48 mg/g) and peroxide value (from 0.156 to 0.180 g/100g) after 20 days of storage indicates mild lipid hydrolysis and early-stage oxidation, both within acceptable industry standards. These results suggest that the oil resists rapid degradation and retains functional integrity over time. The enhanced stability in antioxidant-enriched formulations further validates the protective role of tocopherols and polyphenols. These compounds delay oxidation by scavenging peroxyl radicals and stabilizing unsaturated fatty acids. The inclusion of antioxidants is thus crucial not only for nutritional preservation but also for ensuring product quality in long-term commercial applications (Meng et al., 2023).

Conclusion

This study comprehensively investigated the extraction efficiency, biochemical composition, antioxidant potential, oxidative stability, and sustainability attributes of peony seed oil (PSO), offering novel insights into its value as functional oil. The findings underscore the superiority of ultrasound-assisted extraction (UAE) over conventional techniques, not only in maximizing yield but also in preserving the integrity of thermolabile bioactive compounds such as polyphenols and tocopherols. UAE demonstrated remarkable advantages in terms of energy efficiency, environmental safety, and compound retention highlighting its suitability for clean-label production of high-value plant oils. Peony seed oil was found to be exceptionally rich in α-linolenic acid (ALA), constituting over 40% of its total fatty acid profile. This high omega-3 content, in combination with balanced levels of omega-6 and omega-9 fatty acids, positions PSO as a potent dietary lipid capable of supporting cardiovascular, neurological, and anti-inflammatory health. Furthermore, the oil’s strong antioxidant activity, as demonstrated by DPPH, ABTS, and FRAP assays, and its relatively low acid and peroxide values during extended storage, underscore its oxidative stability and suitability for long-term use in food, nutraceutical, and cosmetic formulations. The synergistic effects observed between tocopherols and polyphenols further strengthened the oil’s resistance to lipid peroxidation, offering a scientifically sound rationale for co-enrichment strategies in antioxidant formulations. The results suggest that PSO can serve as a functional ingredient and a natural preservative, contributing to improved product shelf life and reduced dependence on synthetic additives. Beyond its compositional merits, the study highlights peony cultivation as an environmentally resilient and economically valuable option for sustainable agriculture. Peonies can thrive on marginal lands with minimal water input, making them ideal for climate-adapted farming systems. This is particularly relevant for countries aiming to diversify oilseed production while conserving arable land for staple crops. Peony's low-input cultivation model, when integrated with green extraction technologies like UAE, offers a pathway to simultaneously address issues of food security, rural income generation, and ecological conservation. This study provides a compelling case for the industrial-scale adoption of UAE for peony seed oil production and supports the broader use of PSO as a bioactive-rich, health-promoting, and shelf-stable oil. Future work should focus on process optimization at scale, clinical validation of its health effects, and integration into value-added functional food systems. The convergence of nutritional excellence, environmental sustainability, and technological feasibility makes peony seed oil a promising contributor to the next generation of functional and sustainable food products.

Significance Of The Study:
To enhance oil yield while ensuring maximum recovery of bioactive constituents, an extensive account is provided on the optimization of peony seed oil extraction via ultrasonic –assissted extraction (UAE). Besides discussing the enhanced antioxidant properties and nutritional value of peony seed oil maximally extracted under all identified conditions, the study further adds to the body of knowledge on the RSM –aided process optimization. Thus, this information is helpful for functional foods and nutraceutical applications as well as for such oils marketed as health-promoting edible oil. The study aims at the development of peony seed extraction techniques that are economical and cost- effective for realization in the food and health sectors , the foreseen peony seed trade ,and high –end natural product marketing.

Conflict of Interests and Compliance with Ethics Guidelines:
The authors declare no conflict of interests.

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Contribution of Authors:
Original draft, Conceptualization, investigation, and writing (review and editing) of this research were conducted by Nabila Jabbar. Project management and supervision of the research were handled by Li Gao. Formal analysis of the paper was performed by Haixia Ji. Methodology, software, and resources were managed by Zhengxuan He. The original draft of the work was written by Nabila Jabbar.

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