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Research Paper

Effects of Different Extraction Methods on the Quality of Peony Seed Oil and Its Emulsion Properties

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Abstract: Peony seed oil, as a functional oil rich in ω-3 polyunsaturated fatty acids and natural antioxidant components, has its extraction process directly affecting the maintenance of bioactive substances and the added value of the product. In this analysis, the effects of wet milling-micronized continuous leaching method (MCI), solvent extraction (SE), soxhlet extraction (SOXE) and ethanol-assisted enzymatic hydrolysis (EAH) on the quality of peony seed oil were comprehensive quality assessment, and the emulsification process was optimized. Oil production efficiency and key physicochemical parameters, acid value, peroxide value, saponification value, and iodine value, as well as bioactive compounds including β-sitosterol, γ-tocopherol, and squalene, fatty acid composition and functional properties (DPPH radical scavenging) and emulsification properties of peony seed oil were analyzed. It was shown that the MCI method yielded the highest oil yield (34.77%), which was 12.3%, 18.6% and 25.4% higher than those obtained by SE, SOXE and EAH methods, respectively. In addition, micronized continuous leaching method MCI significantly retained thermosensitive components such as α-linolenic acid (48.07%), β-sitosterol (948.66 mg/kg), γ-tocopherol (543.92 mg/kg) and squalene (130.88 mg/kg). To evaluate the emulsification properties, emulsions were prepared with different concentrations of emulsifiers emulsions were prepared. The emulsion stability was best at an emulsifier concentration of 15%, the particle size was concentrated at 0.5-3.5 μm, the viscosity showed a positive correlation with concentration, while the variation in zeta potential indicated a combined stabilization effect of electrostatic repulsion and steric hindrance. In conclusion, this study pioneered the low-temperature short-time MCI technology, which broke the bottleneck of traditional extraction and solvent methods in destroying the heat-sensitive components and constructed the "extraction-emulsification" process system, establishing a theoretical foundation for enhanced utilization of peony seed oil in functional foods and cosmetic formulations. These research results provide key technological breakthroughs for the high-value application of peony seed oil.

Keywords: Peony Seed Oil; Wet Milling-Micronized Continuous Leaching; Alpha-Linolenic Acid; Emulsion

Introduction

Paeonia (Ranunculaceae), commonly known as the "King of Flowers" or China's national flower, holds profound cultural and historical significance. Peony seeds, consisting of both seed hulls and kernels, serve as the raw material for extracting peony seed oil through specialized extraction techniques. In 2011, the National Health Commission of China officially recognized peony seed oil, a natural plant-based lipid, as a new source of edible oil. marking its integration into food industry and consumer markets (Wang, Su et al. 2023). Notably, this oil is distinguished by its exceptionally high α-linolenic acid (ALA) content (>41% of total fatty acids), substantially exceeding conventional vegetable oils (e.g., rapeseed oil: 8-10%; soybean oil: 6-8%) (Cao, Wang et al. 2022). As

an essential ω-3 polyunsaturated fatty acid, ALA functions as the metabolic precursor compounds for eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) were utilized, demonstrating critical biofunctions in lipid metabolism modulation, anti-inflammatory responses, cardiovascular improvement, and neuroprotection (Zhu, Fan et al. 2024). Furthermore, peony seed oil contains micronutrient activators including tocopherols (vitamin E), phytosterols, and squalene: tocopherols exhibit antitumor potential via free radical scavenging and apoptosis regulation (e.g., suppressing HepG2 hepatocellular carcinoma proliferation) (Deviren and Aydin 2023); phytosterols competitively inhibit cholesterol absorption, reducing serum LDL levels (Echenique, Alvarez-Rivera et al. 2024). Squalene enhances oxygen metabolism efficiency, mitigating ischemia-reperfusion injury (Cheng, Ji et al. 2024). These attributes underscore peony seed oil's prominent nutritional and therapeutic value, warranting further investigation into its bioactive synergies. Recent studies have further shown that natural plant extracts can synergistically regulate lipid metabolism through multiple targets. For example, the ethanolic extract of Acehnese Lime (Citrus aurantifolia) bark significantly reduced serum LDL-C levels in hypercholesterolemic model rats (by 28%) by inhibiting HMG-CoA reductase activity(Sitio, Akmal et al. 2024). In addition, the flavonoid component of the extract reduced lipid peroxidation damage, which complemented the cholesterol uptake inhibitory effect of sterols in peony seed oil, suggesting that peony seed oil may have the ability to intervene in metabolic diseases. Therefore, there is an urgent need to develop a safe, efficient and low energy consumption peony seed oil extraction technology to meet its high demand.

Oil extraction technologies critically influence product quality and functional compound integrity (Han, Liu et al. 2018). Despite the advances in extraction techniques, there are still unresolved challenges hindering the productionization of peony seed oil. Conventional solvent extraction, despite high efficiency, raises safety concerns due to volatile, flammable, and toxic organic solvents (Gao, Zhou et al. 2024). Soxhlet extraction, though operationally simple, induces thermal degradation of heat-sensitive compounds (e.g., tocopherols, polyphenols) through prolonged high-temperature reflux (>60°C) (S., Debabrata et al. 2021). Ethanol-Assisted Aqueous Enzymatic Hydrolysis, despite eco-friendly advantages, faces challenges in enzyme activity suppression (e.g., 30-50% reduction in cellulase activity) within ethanol-water biphasic systems, leading to inconsistent extraction yields (Song, Zhang et al. 2019). In contrast, MCI can crush the material down to the micron level and combine it with dynamic countercurrent extraction techniques to maximize the solvent-material interface contact. In addition, it is a room temperature extraction, which avoids oxidative degradation of many heat-sensitive components. Therefore, it is expected that this will become an alternative technology for extracting peony seed oil.

Introducing MCI for the first time in peony seed oil extraction, this study conducted a systematic comparison of its mechanism against SE, SOXE, and EAH regarding oil quality. The comparative assessment of oil extraction efficiency, physiochemical characteristics active ingredients, fatty acid profile and concentration, and emulsification characteristics enabled the selection of the best extraction technique. These findings provide an innovative solution for the green and efficient preparation of peony seed oil. Subsequent research and applications should prioritize further process improvements and broadening the scope of applications.

Materials and Methods

Materials

Peony seeds were sourced from Heze in Shandong Province. Chemical reagents including petroleum ether, hexane, potassium hydroxide, ethanol, methanol, chloroform, foraminol, and sodium carbonate were procured from Sinopharm Chemical Reagent Co., Ltd. Biochemical reagents such as cellulase, 1,1-diphenyl-2-trinitrophenylhydrazine (DPPH), and 2,6-di-tert-butyl-4-methylphenol (BHT) were sourced from Shanghai Biochemical Technology Co., Ltd. Standard references (soy sterol, β-sitosterol, campesterol, γ-tocopherol, α-tocopherol, δ-tocopherol, squalene, and fatty acid standards) were acquired from Shanghai Yuanye Biological Co., Ltd. All other chemicals in this study were of analytical grade and employed without further purification.

Peony Seed Pretreatment

Prior to oil extraction, peony seeds underwent sequential pretreatment procedures: Initially, the seeds were mechanically decorticated and subjected to convective dehydration in a laboratory oven (60°C, 6 h). Allow the temperature to cool to room temperature, then fractionated through a 60-mesh standardized sieve (250 μm aperture). The resultant homogenized powder was aseptically packaged in hermetic containers and stored under refrigeration at 4°C for further analytical processing.

Extraction Method of Oil

This study employed a comparative design to systematically assess how MCI, SE, SOXE, and EAH extraction methods impact peony seed oil quality, with the objective of identifying the optimal technique.

MCI: Accurately weighed 50 g of peony seed powder was pre-treated with micronization in a high efficiency pulverizer and added to 250 mL of n-hexane solvent at a material-liquid ratio of 1:5 (g/mL). The mixture was thoroughly homogenized and transferred to an extraction device for continuous leach over 40 min. After extraction, the solution underwent vacuum distillation using a rotary evaporator to remove the organic solvent, yielding crude peony seed oil. The collected oil was precisely weighed, sealed in light-blocking containers, and preserved at -4°C for future applications.

EAH: Following the methodology established in reference Zhang, Yu et al. (2024): 20 g portion of peony seed powder was precisely weighed and homogenized in 140 mL of 95% ethanol, yielding a solid-to-liquid ratio of 1:7. Cellulase enzyme (150 mg) was introduced into the solution, followed by pH adjustment to 9.0. Enzymatic hydrolysis was conducted at a constant temperature of 60°C for 3 hours. Subsequently, the sample was centrifuged at 5000 rpm for ten minutes to separate the crude emulsion. A dual-phase demulsification protocol was implemented, consisting of low-temperature freezing at -20°C for 18 h followed by thermal-assisted demulsification in a 60°C water bath for 1 h. Secondary centrifugation was performed, and the separated oil phases were combined. The purified oil was dried, allowed to equilibrate to ambient temperature, measured for mass, and preserved at -4°C.

SE: Following the modified protocol adapted from (Zhang, Shuai et al. 2023). Peony seed powder (10 g) was precisely weighed and combined with 2 mL of 4 M HCl. The acid hydrolysis process was carried out at 80°C in a thermostatic water bath for 3 h, with vortex mixing every 30 min. After cooling the hydrolysate to 25°C, chloroform (4 mL) and methanol (2 mL) were added sequentially in a 2:1 v/v ratio. The mixture was thoroughly shaken for 15 min with a mechanical shaker, subsequently subjected to centrifugation at 3000 revolutions per minute for five minutes. The chloroform-enriched lower phase was collected via separatory funnel and concentrated using a nitrogen evaporator. The obtained oil was dried under vacuum, cooled to ambient temperature, weighed, and stored at -4°C.

SOXE: Based on the method of Jinoni, Benjamin et al. (2024) with modifications. Peony seed powder (10 g) was precisely weighed, wrapped in filter paper, and loaded into a Soxhlet extractor. Hexane (100 mL) was added, and the extraction process was maintained continuously at 65°C for seven hours. The extract was concentrated to constant mass using a rotary evaporator (50°C water bath, -0.1MPa vacuum). The obtained oil was dried under vacuum, brought to room temperature, weighed, and stored at −4°C.

Measurement of Oil Yield

Oil Yield (%) = [Quality of peony seed oil obtained/ Quality of peony seed powder] ×100%

Determination of Physical and Chemical Properties

The quality parameters of peony seed oil were determined following AOCS official methodologies: Saponification Value (SE, Cd 3-25), Peroxide Value (POV, Cd 8b-90), Acid Value (AV, Cd 3d-63 via potentiometric titration), and Iodine Value (IE, Cd 1d-92 using Wijs solution method).

Sterol Analysis

Sterols content was adapted from Azadmard-Damirchi, Habibi-Nodeh et al. (2010).Peony seed oil (0.5 g) was subjected to alkaline saponification by mixing using 20 milliliters of 0.5 molar NaOH -ethanol solution. The solution was maintained at 70°C under reflux condensation for 60 minutes. Upon reaching ambient temperature, 15 mL of saturated NaCl solution and 30 mL of petroleum ether were added in sequence. The solution was then vortexed vigorously for 30 seconds to induce phase separation. The supernatant organic layer was carefully retrieved, and the liquid-liquid extraction was performed three times. Combined organic fractions were dehydrated through an anhydrous Na_2SO_4-packed glass funnel. The extract was concentrated via vacuum distillation (rotary evaporator, 60°C) and reconstituted in HPLC-grade methanol. Prior to analysis, the mixture was subsequently filtered through 0.45 μm PTFE membrane filters. The chromatographic analysis was performed on a C18 reversed-phase analytical column (250 mm × 4.6 mm internal diameter, 5 μm particle size) employing an isocratic mobile phase system (methanol/water 80:20, v/v) with a flow rate maintained at 1.5 mL/min. Sample introduction volume was set to 15 μL while keeping the column oven temperature constant at 35°C. UV detection was conducted at 210 nm wavelength. Sterols were identified by retention time matching against certified standards, with 5α-cholestane as internal standard for quantification.

Tocopherol Analysis

The method of reference Gao, Zhang et al. (2024) was performed. Homogenize 1 g peony seed oil with 0.1 g butylated hydroxytoluene (BHT) to prevent lipid oxidation during analysis. Add 10 mL acetonitrile (HPLC-grade) and subject to sonication-assisted extraction (40 kHz, 30 min). Volumetric Adjustment: Dilute the extract to 25 mL with acetonitrile in a volumetric flask and vortex-mix for 1 min. Filter through 0.45 μm PTFE membrane filters to remove particulate matter prior to injection. Chromatographic analysis was performed on a reversed-phase C18 column (250 × 4.6 mm, 5 μm) using an isocratic mobile phase of acetonitrile/water (95:5, v/v) at a flow rate of 1.0 mL/min, with the column temperature maintained at 40°C.A 15 μL injection volume was used, and fluorescence detection was employed (Excitation at 294 nm, Emission at 328 nm).

Squalene Analysis

Adapted from Azlina, Hong-Yeng et al. (2023) with modification. 1.0 g of peony seed oil was combined with 15 mL of a 2% (w/v) KOH-methanol solution. Alkaline hydrolysis was carried out at 65°C for 50 min under reflux condensation until the lipid droplets were fully cleared. The saponified mixture was then cooled to 25°C, followed by the successive addition of 10 mL deionized water and 10 mL n-hexane. The solution was vortexed vigorously for 30 s for phase separation. The supernatant organic layer was isolated, and the extraction procedure was replicated thrice. The hexane fractions were then pooled and concentrated to dryness using vacuum rotary evaporation at 60°C. The residue was re-dissolved in HPLC-grade methanol and then passed through 0.45 μm PTFE filters before injection.

The chromatographic separation was conducted using a C18 reversed-phase analytical column (250 mm length × 4.6 mm i.d, 5 μm particle size) employing an acetonitrile/methanol mobile phase (60:40, v/v) at 1.0 mL/min flow rate. The separation was conducted at 40°C column temperature, with detection at 210 nm wavelength and 10 μL sample injection volume.

Quantification of Fatty Acid Composition

The approach of Feng, Wang et al. (2025) was adapted and optimized for the methylation process. Two grams of peony seed oil were mixed with 1 mL of 10% (v/v) hydrochloric acid-methanol solution and incubated in a water bath at 60°C for 3h, with vortexing every 30 min. Upon cooling to 25°C, 2 milliliters of n-hexane and 1 milliliter of a saturated sodium chloride solution were introduced. The resulting mixture underwent vigorous shaking for 15 min, followed by centrifugation at 3,000 times gravitational force for a duration of 5 min. The upper organic phase was separated, and 1 mL was filtered through a 0.45 μm membrane filter for gas chromatography analysis. A HP-88 capillary column (100 m length × 0.25 mm internal diameter × 0.2 μm film thickness) was utilized to conduct the chromatographic separation, with a 10:1 split injection, an inlet temperature of 250°C, and a carrier gas flow rate of 0.5 mL/min. The temperature program started at 130°C for 5 min, then increased at 4°C/min to 240°C, where it was held for 30 min. The detection system was maintained at 250°C. The identification of fatty acid methyl esters (FAMEs) was achieved through the comparison of their retention times with those of certified standards, while quantification was conducted by means of area normalization.

Antioxidant Capacity

The antioxidant potential was evaluated through the DPPH radical scavenging method. The experimental protocol was adapted from Yang, Zhang et al. (2017). Peony seed oil 5.00 g was accurately weighed and subjected to stepwise gradient dilution in anhydrous ethanol to prepare serial concentrations (10-50 mg/mL). Subsequently, an equal volume (2.0 mL) of freshly prepared DPPH working solution was added to 2.0 mL of the test solution (0.05 mg/mL in 95% ethanol) under light-protected conditions (25°C, 30 min). A UV-Vis spectrophotometer measured absorbance (A_1) at 519 nm, with parallel controls included: blank controls (DPPH solution + ethanol, A_0) and sample background controls (sample solution + ethanol, A_2), with vitamin C solution at equivalent concentrations serving as positive reference. All solutions were prepared immediately before use, and experimental operations were conducted under strict light-exclusion conditions.

The formula for calculating the clearance rate is as follows:

$$\text{DPPH free radical scavenging (\%)} = [(1-(A_1-A_2)/A_0)] \times 100\%$$

Determination of Emulsification Stability of Peony Seed Oil

Preparation of emulsions: The method was slightly modified according to Yao, Huang et al. (2025). The experimental procedure was as follows: Blend Tween 20 (Polysorbate 20, 5-25% w/w) with peony seed oil under thermostatic conditions (60°C water bath) with continuous vortex mixing for 20 min until homogenization. Pre-heat deionized water to the oil-phase equilibrium temperature (60°C). Inject the oil phase into the aqueous phase at 1 mL/min through a precision peristaltic pump, followed by high-shear dispersion

(12,000 rpm, 5 min) using a rotor-stator homogenizer to form a crude emulsion. Process through two-stage high-pressure homogenization: primary pass at 40 MPa (400 bar); secondary pass at 5 MPa (50 bar). The peony seed oil emulsion was prepared and stored for subsequent use.

Measurement of emulsion viscosity: Viscosity analysis protocol was adapted from reference Choi and Yoo (2009). A rotational viscometer was employed to measure the apparent viscosity of emulsions under standardized rheological conditions: temperature-controlled at 25.0°C, spindle rotation speed at 120 rpm, with continuous measurement over 15 min to ensure steady-state flow behavior.

Emulsion samples underwent zeta potential and particle size analysis: Referring to the method of Yin, Deng et al. (2012). The zeta potential and particle size distribution of emulsions containing varying emulsifier mass fractions (5-25% w/w) were analyzed using a laser diffraction particle size analyzer under standardized conditions.

The emulsification the activity index (EAI) and emulsion stability index (ESI) were evaluated through the following procedures: The method of N. and E. (2002) is cited with minor modifications. Dilute 0.5 mL freshly prepared peony seed oil emulsion (Section 2.9.1) 100-fold with 0.1% (w/v) sodium dodecyl sulfate (SDS) solution. Vortex for 30 s to ensure homogeneity (A_0). Initial absorbance: Measure immediately at λ = 500 nm using SDS solution as blank. Time-dependent absorbance: Repeat after 10 min static incubation (A_{10}). The following protocol was used to determine the Emulsifying Activity Index (EAI) and Emulsion Stability Index (ESI):

$$EAI = 2\times2.303/C\times(1-ø)\times10^4\times A_0\times N$$

$$ESI = A_{10}/A_0\times100\%$$

Where: C is the concentration of the emulsion (g/mL), ø is the volume fraction of the oil phase, Where A_0 denotes sample absorbance at time zero, N represents dilution count, and A_{10} indicates absorbance after 10 min of placement.

Data Analysis

Statistical analysis was conducted that experimental data for hypothesis testing was performed with SPSS 22.0, and visualization was conducted using Origin Pro 8.5. Triplicate independent experiments generated datasets subjected to normality verification and homogeneity of variance assessment. Data are reported as mean ± standard deviation (SD).

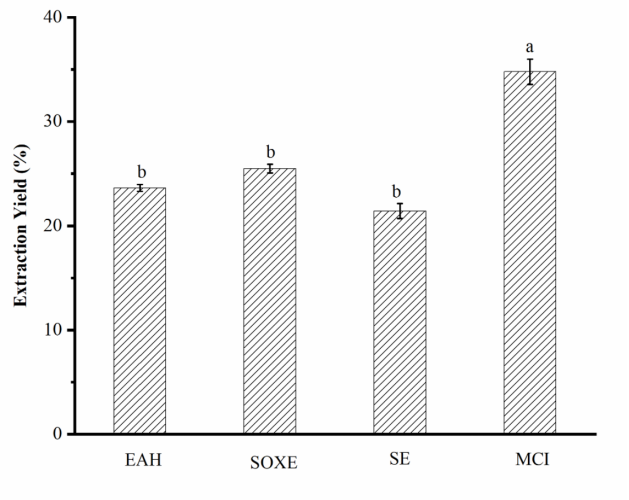

Fig. 1. Oil yield from peony seed oil obtained by different extraction methods were compared.

Results and Discussion

Impacts of Various Extraction Techniques on the Production Extraction Efficiency of Peony Seed Oil

For the purpose of reflect the ability of the extraction process to release oils from the feedstock. Four distinct extraction processes were employed to evaluate their impact on peony seed oil yield: MCI, SOXE, EAH and SE. As shown in Fig. 1, MCI sample demonstrated a significant advantage, achieving an oil yield of 34.77% among the four extraction methods for peony seed oil. This represents increases of 36.3%, 47.2%, and 62.4% compared to SOXE (25.49%), EAH (23.64%), and SE (21.41%), respectively. The differences in oil extraction rates among methods align with the findings of Tarhan (2021). The superior extraction efficiency of MCI may be attributed to the continuous solvent-material contact, ensuring thorough solubilization and facilitating lipid release, thereby enhancing oil yield. The ethanol-water solvent combination mimics organic solvents in oil extraction, particularly in oil-rich plant materials. Due to its comparable solubilization efficiency to SOXE, the oil yields of these two methods are relatively close (Hu, Xi et al. 2021). The inefficiency of SE likely stems from incomplete solvent penetration or cell wall disruption, preventing full lipid release and reducing oil yield (Cao, Wang et al. 2022), This is very close to the oil yield (20.78%) of Pall seed oils extracted by solvent extraction method by Nie, Zhang et al. (2020).

Table 1. The physicochemical characteristics of peony seed oil derived from various extraction techniques were evaluated.

Physiochemical properties	MCI	EAH	SOXE	SE
IV (g/100g)	171.42±0.84[a]	170.51±0.82[a]	169.64±0.51[a]	170.5±0.79[a]
SV (mgKOH/g)	190.98±1.86[a]	187.3±0.63[b]	186.84±1.54b	182.5±1.16[c]
AV (mgKOH/g)	1.64±0.41[c]	1.83±0.35[ab]	1.77±0.34[b]	1.93± 0.38[a]
POV (mmol/kg)	2.98± 0.22[b]	4.94±033[a]	5.51±0.36[a]	6.2±0.49[a]

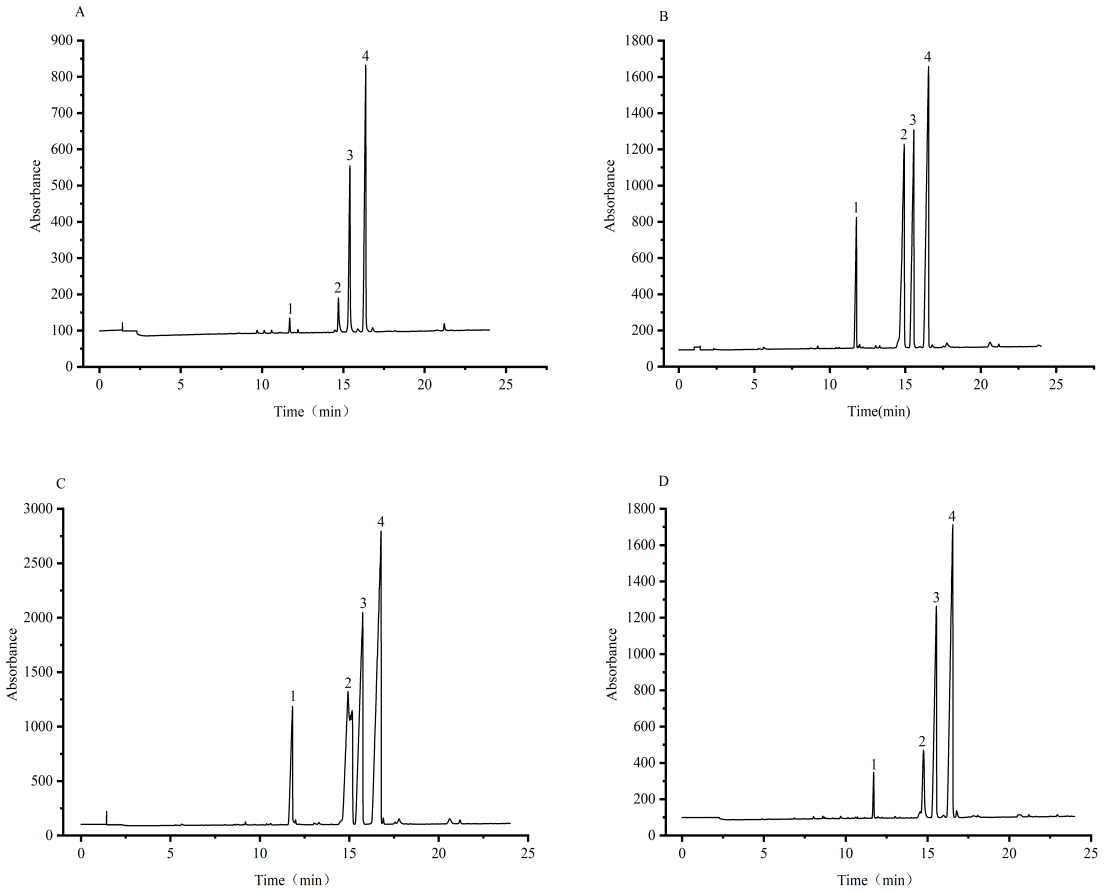

Fig. 2: GC profiles of fatty acids extracted from peony seed oil by four different methods of (A) EAH; (B) SE; (C) SOXE; (D) MCI**.** **(**1) palmitic acid, (2) oleic acid, (3) linoleic acid, (4) alpha-linolenic acid

Physicochemical Characteristics of Peony Seed Oil Extracted by Various Methods

Lipid quality and chemical characteristics from various extraction methods were evaluated using iodine value (IV), peroxide value (POV), acid value (AV), and saponification value (SV). The IV quantifies the degree of unsaturation in oils, the number of double bonds in the carbon chain of the oils (Cui, Wei et al. 2025). Peroxide value is used to determine the amount of peroxide in oils, which is one of the products of oxidation reactions in oils(Run-Yang, Hua-Min et al. 2019), measure the amount of free fatty acids in oils (Moumen, Mansouri et al. 2015). Saponification value is used to indicate the molecular size of oils and the ability of fats and oils to react with alkalis to form soap (Uoonlue and Muangrat 2019).

As presented in Table 1, the peony seed oil obtained through four different extraction techniques exhibited slight variations in iodine value (169.64-171.42g/100g), saponification value (182.5-190.98 mg KOH/g), acid value (1.64-1.93 mg KOH/g), and peroxide value (2.98-6.2 mmol/kg). However, all parameters complied with national standards. Due to short solvent-material contact time, SE preferentially extracted low-molecular-weight

compounds (e.g., free fatty acids),As these contribute minimally to saponification value, SE showed a lower saponification value (182.56 mg KOH/g) (Conte, Gullich et al. 2016). In contrast, MCI achieved full lipid extraction through prolonged solvent contact, producing a substantially greater saponification value (190.98 mg KOH/g) compared to alternative techniques. The acid value (indicating free fatty acid content per gram of oil) of MCI sample was 1.64 mg KOH/g, demonstrating superior quality. This resulted from MCI's low-temperature/short-duration operation, which minimized triglyceride decomposition and free fatty acid generation. Peroxide value (reflecting oxidation status), a critical edible oil quality parameter (Li, Zhu et al. 2025), remained low due to MCI's rapid extraction that reduced oil exposure time.

Fatty Acid Profile of Peony Seed Oil Extracted Through Various Approaches

Fatty acid composition determines the physicochemical properties of oils, which in turn affects their application in food, cosmetics or industry. GC analysis was employed to assess the fatty acid composition and relative quantities in peony seed oils obtained via four extraction methodologies. As shown in Fig. 2, the main fatty acids in the oils from the four methods were palmitic acid, oleic acid, linoleic acid, and α-linolenic acid. The fatty acid compositions of the oils obtained by these methods did not show significant differences, which aligns with prior studies (Chang, Wang et al. 2020).

Extraction techniques differentially modified peony seed oil's nutritional composition (Table 2). The experimental data showed that MCI extracted peony seed oil was of the highest quality, with a total unsaturated fatty acid content of 96.98%, which was significantly higher than those extracted by SE (91.23%), SOXE (89.99%) and EAH (87.91%). Remarkably, α-linolenic acid levels in MCI rose significantly to 48.07%, which was slightly higher than that of α-linolenic acid in peony seed oil extracted by cold pressing method (44.06%) by Wang, Xu et al. (2023). This advantage is mainly attributed to the mild environmental conditions of the MCI process, which can effectively alleviate the lipid peroxidation reaction and maintain the structural integrity of heat-sensitive α-linolenic acid.

Table 2: Evaluation of fatty acid profiles in peony seed oils produced by various extraction techniques

Fatty Acids (%)	MCI	EAH	SOXE	SE
C16:0	6.38%± 0.13^{ab}	6.45%± 0.95^{a}	5.79%± 0.22^{b}	5.97%± 0.74^{ab}
C18:1	22.68%± 0.53^{a}	20.95%± 0.8^{a}	21.71%± 0.73^{a}	21.47%± 0.65^{a}
C18:2	26.23%± 0.62^{a}	24.12%± 1.01^{a}	24.91%± 0.73^{a}	25.23%± 0.35^{a}
C18:3	48.07%± 0.43^{a}	42.85%± 0.59^{b}	43.37%± 0.24^{b}	44.52%± 0.61^{b}
MUFA	22.68%± 0.53^{a}	20.95%± 0.8^{a}	21.71%± 0.73^{a}	21.47%± 0.65^{a}
PUFA	74.35%± 1.05^{a}	66.95%± 1.6^{b}	68.28%± 0.97^{b}	69.73%± 0.96^{b}
UFA	96.98%± 1.58^{a}	87.91%± 2.4^{b}	89.99%± 1.7^{ab}	91.23%± 1.61^{b}

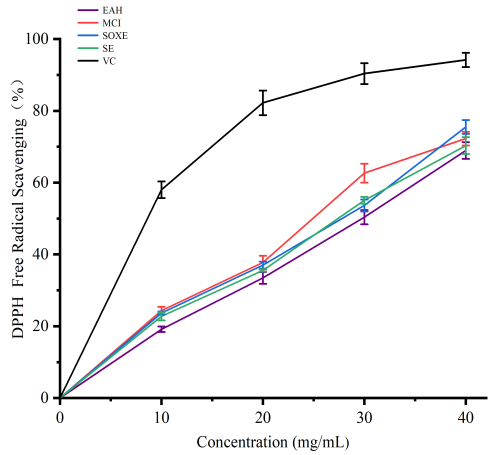

Fig. 3. Mass concentration-effect relationship of different extraction methods on the scavenging of DPPH free radicals

Antioxidant capacities of peony seed oils obtained through different extraction methods were compared

DPPH is a chemical reagent commonly used to determine antioxidant activity (Marecek, Mikyska et al. 2017). The DPPH antioxidant assay allows preliminary verification of the presence of antioxidant components in the oil and assessment of the strength of its antioxidant properties (Li, Zhu et al. 2025). As depicted in Fig. 3, the DPPH free radical scavenging ability increased progressively with rising concentrations. At standardized concentrations, the four methodologies exhibited similar efficiencies in DPPH radical neutralization. This observation may be attributed to the extraction of comparable antioxidant compounds, whose radical scavenging activities are intrinsically stable, thereby minimizing the influence of extraction technique on DPPH scavenging efficiency. This result aligns with the findings of reference which reported that different solvents had limited impact on the antioxidant performance of Bischofia polycarpa seed oil (Wang, Su et al. 2023). The maximum scavenging activity of peony seed oil was achieved at a concentration of 40 mg/mL, with SE (75.51%) > MCI (72.26%) > SOXE (70.32%) > EAH (68.93%). While peony seed oil exhibited lower antioxidant capacity compared to conventional antioxidant Vc, its natural source properties and considerable free radical scavenging capacity (peak height >68%) still demonstrated the value of its development as a functional natural antioxidant. This finding is in line with the trend of the green coffee bean extract study by Duangjai, Rawangkan et al. (2024): natural extracts are not as good as synthetic antioxidants (e.g., BHT) but have potential for application due to their high safety profile and multi-targeted effects (e.g., activation of the Nrf2 pathway).

Table 3: Evaluation of tocopherol levels in peony seed oils produced using various extraction techniques

Compounds	MCI	EAH	SOXE	SE
α-Tocopherol (mg/kg)	64.17 ±1.612^{a}	52.42± 1.25^{b}	60.41± 1.17^{b}	60.11± 2.23^{a}
γ-Tocopherol (mg/kg)	543.92± 5.78^{a}	376.7± 3.09^{b}	513.83± 4.12^{a}	412.85± 4.27^{b}
δ-Tocopherol (mg/kg)	18.81± 1.16^{a}	16.98± 1.94^{ab}	15.03± 1.61^{bc}	13.61± 2.23^{c}

Tocopherol Levels Ii Peony Seed Oil Produced Using Various Extraction Techniques

γ -Tocopherol is a lipid-soluble antioxidant that is strongly linked to the unsaturated fatty acids found in vegetable oils. As illustrated in Table 3, the tocopherol content of peony seed oil varied notably depending on the extraction method used. γ-Tocopherol predominated (85.2-92.3% of total tocopherols), followed by α- and δ-tocopherols. Similarly Chang, Wang et al. (2020) studied the study on peony seed oil from five distinct regions revealed that γ-tocopherol accounted for over 85% of the total tocopherols.

The highest overall tocopherol levels were observed in the oil extracted using the MCI. This may be attributed to MCI's ability to effectively pulverize the seed material into finer particles, this enhances the interfacial contact area between the lipid phase and bioactive constituents. Such structural disruption not only improves oil yield but also facilitates the efficient release and retention of γ-tocopherol, resulting in its notably high concentration in the final product.

Table 4: Evaluation of sterol and squalene levels in peony seed oils produced using various extraction techniques

Compounds	MCI	EAH	SOXE	SE
Stigmasterol (mg/kg)	61.72± 2.02^{a}	38.58± 1.97^{b}	53.11± 0.72^{a}	29.29± 1.13^{b}
Campesterol (mg/kg)	105.24± 0.54^{a}	91.01± 0.47^{b}	88.47± 0.32^{b}	102.3± 0.63^{a}
β-sitosterol (mg/kg)	948.66± 1.78^{a}	859.21± 1.02^{b}	805.35± 0.72^{c}	795.3± 2.31^{c}
Squalene (mg/kg)	130.88± 1.46^{a}	96.77± 1.76^{b}	84.78± 2.69^{c}	80.78± 0.78^{b}

Sterols and squalene in peony seed oil extracted by different methods

Phytosterols, a class of bioactive compounds with demonstrated cholesterol-lowering and anti-inflammatory properties (Zhang, Zhan et al. 2025). Table 4

demonstrates that the sterol content in peony seed oil differed based on the extraction method used. Three types of phytosterols were identified: stigmasterol, campesterol, and β-sitosterol. Notably, β-sitosterol exhibited the highest abundance, displaying concentration levels between 795.33 and 948.66 mg/kg, followed by campesterol (88.47 105.24 mg/kg). Consistent with the study of Cao, Wang et al. (2022): The content of β-sitosterol was considerably higher than that of other sterol compounds, making up over 80% of the total sterol content. The slightly elevated sterol content observed in the oil extracted by the MCI technique may be attributed to the micro pulverization process, which disrupts the seed cell walls and facilitates the release of intracellular components, thereby enhancing sterol extraction efficiency (Wang, Zheng et al. 2025). The process characteristics provide a theoretical basis for optimizing the extraction of plant active ingredients.

Squalene, a bioactive triterpenoid hydrocarbon with demonstrated antioxidant and chemopreventive properties (Beltrán, Bucheli et al. 2016). As shown in Table 4, all methods yielded squalene concentrations (84.78-130.88 mg/kg) exceeding the minimum requirement (≥50.0 mg/kg) stipulated in the National Standard for Peony Seed Oil (GB/T 40622-2021, Section 5.3.2), validating their efficacy in preserving lipid-soluble bioactive constituents. Similarly Lubna (Masoodi, Gani et al. 2025) obtained walnut oil by different extraction methods, the solvent extraction method extracted the oil with lower content of squalene. Indicates that MCI is more suitable for the extraction process of peony seed oil.

Analysis of EAI and ESI

EAI indicates the role of the emulsifier in enhancing the oil-water interfacial activity during the emulsification process, while ESI demonstrates the emulsion's ability to sustain the oil-water dispersion during storage or when subjected to external disturbances. EAI and ESI are important parameters for assessing the quality of the formation emulsions (Cheng, Xiong et al. 2009). As depicted in Fig. 4, the emulsions showed initial increases in both EAI and ESI, then decreased as the emulsifier's mass fraction increased. Within the range of 5% to 15%, the progressive addition of emulsifier enhanced the number of adsorption sites at the oil-water interface, facilitating the formation of a more robust interfacial film, thereby improving both emulsification efficiency and emulsion stability. However, when the emulsifier concentration was further elevated to 20% and 25%, a decline in EAI and ESI was observed, likely due to interfacial film destabilization, droplet aggregation, and disruption of the emulsion's structural integrity (Shi, Li et al. 2017). Tsuda, Iida et al. (2025) studied high molecular weight emulsifiers for lentil seeds and found that emulsions prepared with low molecular weight fractions exhibited increased aggregation and agglomeration of oil droplets. In contrast, high molecular weight fractions enhanced interface stability, but excessively high fractions resulted in thicker interfacial films, which also reduced EAI and ESI. Their study further confirmed that the optimal EAI and ESI occurred at a 15% emulsifier mass fraction.

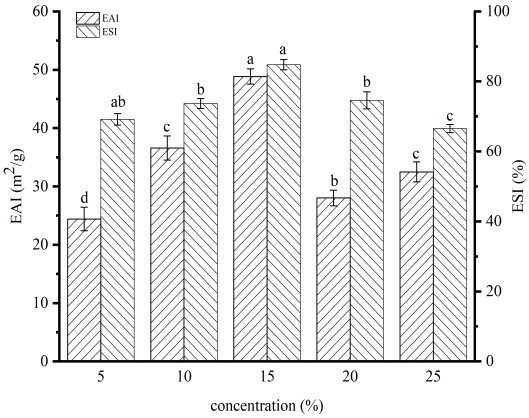

Fig. 4: Effect of different mass fractions of emulsifiers on EAI and ESI of emulsion

Analysis of Emulsion Zeta Potential

In the emulsion system, in addition to spatial stability, electrostatic interactions that these factors play a significant role in maintaining emulsion stability. The absolute value of zeta potential serves as a critical parameter reflecting the strength of electrostatic repulsion between dispersed droplets, thereby providing insight into emulsion stability. As depicted in Fig. 5, emulsions formulated with emulsifier concentrations ranging from

5% to 25% exhibited pronounced negative surface charges. Although Tween 20 is a nonionic surfactant, it can induce negative zeta potentials on emulsion droplets, likely due to interactions with residual anionic compounds present in the formulation system (Zhang, Yan et al. 2021). This charge contributes to the negative zeta potential of the emulsion.

As the emulsifier mass fraction increased, the absolute zeta potential reached its maximum at 20%, then decreased at 25%. When the emulsifier mass fraction was low, insufficient adsorption of emulsifier molecules on oil droplet surfaces led to lower surface charge density and thus a reduced zeta potential (Yin, Deng et al. 2012). Increasing the emulsifier mass fraction enhanced surface

charge density, gradually raising the zeta potential. However, excessively high emulsifier concentrations induced mutual droplet attraction, destabilizing the emulsion and reducing the absolute zeta potential (Tang, Song et al. 2024).

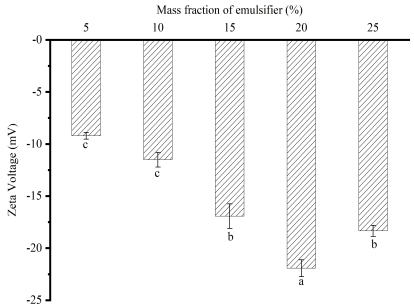

Fig. 5: Effect of different mass fractions of emulsifiers on the zeta potential of emulsions

Analysis of Emulsion Average Particle Size

After preparing emulsions with different emulsifier mass fractions, mean particle size for each emulsion was quantified via laser particle size analysis at days 1 and 30 post-preparation. As demonstrated in Fig. 6, the droplet size of the emulsions exhibited a decreasing-then-increasing trend with rising emulsifier concentration. This is in line with the findings of Hu, Wei et al. (2024) that the viscosity of emulsions shows a tendency to decrease and then increase with increasing different mass fractions of emulsifier.

The smallest average particle size was observed at a mass fraction of 15%, suggesting that at this concentration, the emulsifier provided optimal surface activity, enabling the formation of a sufficient and stable interfacial film. This is the same as the nature of the particle size size of emulsifier viscosity studied by Duanquan, Le-Chang et al. (2022). which shows a tendency to decrease and then increase. This effectively reduced interfacial tension and inhibited droplet coalescence, resulting in finer dispersion. However, increasing the emulsifier concentration further resulted in a gradual increase in droplet size, possibly due to excessive emulsifier molecules enhancing inter-droplet interactions or causing steric hindrance, which in turn promoted aggregation and an increase in particle size (Hu, Wei et al. 2024).

Additionally, the particle size after one month of storage showed a slight increase compared to the initial measurement. This may be attributed to emulsion degradation during storage (e.g., due to temperature, light, oxygen, or time), which weakened emulsification effectiveness.

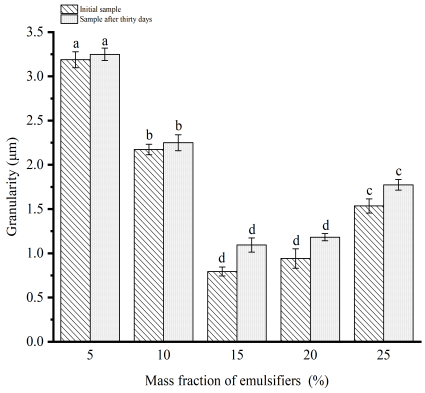

Fig. 6: Effect of different mass fractions of emulsifiers on the mean particle size of emulsions

Analysis of Emulsion Viscosity

As depicted in Fig. 7, the emulsion's viscosity rose as the emulsifier mass fraction increased. High viscosity reduces the Brownian motion of oil droplets and decreases their collision frequency, thereby improving emulsion stability (El-Otmany, El Rhafiki et al. 2021). At a 5% emulsifier mass fraction, insufficient adsorption of emulsifier molecules on oil droplet surfaces led to weak intermolecular interactions and low cohesive forces, resulting in low viscosity. As the emulsifier concentration increased, more molecules were adsorbed on droplet surfaces, forming thicker interfacial films and stronger intermolecular interactions, which significantly increased viscosity. The relative molecular mass is a key factor influencing the viscosity of emulsifiers, with higher relative molecular mass leading to increased viscosity, the higher the viscosity. Consequently, the highest viscosity was observed at 25% emulsifier mass fraction.

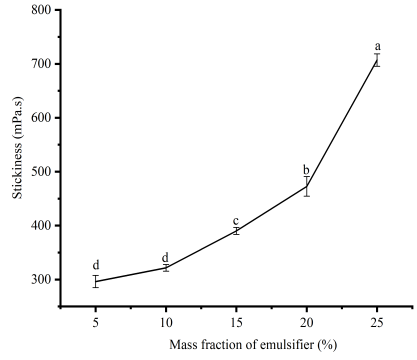

Fig.7: Impact of various mass fractions of emulsifiers on the viscosity of emulsions

Conclusion

In summary, the four extraction methods (EAH, SOXE, SE, MCI) significantly influenced the quality attributes, fatty acid content, and in vitro antioxidant capacity of peony seed oil. Comparative analysis of these methods revealed that MCI outperformed the other techniques in terms of overall extraction efficiency and oil quality. Specifically, the oil obtained through MCI exhibited superior performance across multiple parameters, including oil yield, total fatty acid content (notably α-linolenic acid), and the concentration of bioactive constituents such as γ-tocopherol, β-sitosterol, and squalene. The results indicate that MCI is an efficient approach for improving both the production and nutritional value of peony seed oil, highlighting its potential for large-scale application. Furthermore, the investigation into the emulsification behavior of the oil under varying emulsifier concentrations demonstrated that formulating peony seed oil into emulsions can effectively protect its valuable bioactive components from oxidative degradation, thereby providing a promising strategy for functional product development and industrial utilization. This study confirms MCI as an efficient and eco-friendly extraction technology for peony seed oil, characterized by "low-temperature cell disruption, bioactive retention, and functional enhancement". Future applications should focus on developing health-focused food/cosmetic products and expanding MCI to other oil seeds (e.g., flaxseed, walnut).

In summary, MCI is a promising method for peony seed oil extraction. Further advancements in process optimization and application diversification will be critical for its industrial adoption.

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Author Contributions

Kang Li: Participate in the design of the entire experiment, experimental methodology, data analysis and final paper writing.

Chao Du: Participate in the design of the entire experiment, experimental methodology。

Shuxian Pang: Participate in experimental design and data analysis.

Wu Yang: Participation in experimental programs.

Fengwen Sun: Participation in experimental plans and programs.

Zhaosen Fan: Participation in experimental plans and programs.

Yuanda Song: Participate in the direction and planning of experiments, correcting the format and content of papers.

Ethics

This manuscript presents original, unpublished research, the corresponding author verifies that all co-authors have reviewed, approved, and consented to the final manuscript version. and certifies no ethical issues are involved.

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