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Optimization of perilla seed oil extraction using supercritical CO_2

Suwajee Pothinam, Thanyaporn Siriwoharn, Wachira Jirarattanarangsri*

Division of Food Science and Technology, Faculty of Agro-Industry, Chiang Mai University, Chiang Mai, Thailand

***Corresponding Author:** Wachira Jirarattanarangsri, Division of Food Science and Technology, Faculty of Agro-Industry, Chiang Mai University, Chiang Mai, Thailand. Email: wachira.j@cmu.ac.th

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ORIGINAL ARTICLE

Abstract

This work studied the extraction of perilla oil from perilla seeds using supercritical carbon dioxide, which is valued for its high omega-3 content. Response surface methodology was used to investigate the optimum conditions of perilla oil extraction at pressures of 200, 225, and 250 bar and temperatures of 40°C, 50°C, and 60°C. The fatty acid composition was also investigated. The optimal condition was obtained at 60°C under a pressure of 218 bar. Under this extraction condition, the predicted measures of oil yield (%), acid value, peroxide value, iodine value, saponification value, total phenolic content, DPPH (IC_{50}), ABTS (IC_{50}), and tocopherol were: 37.00%, 1.22 mg KOH/g, 0.94 mEq O_2/kg, 205.00 g I_2/100 g, 196.90 mg KOH/g, 12.14 mg GAE/100 g, 10.93 mg/mL, 154.86 mg/mL, and 604.58 mg/kg, respectively. In addition, gas chromatography analysis indicated that perilla seed oil was primarily composed of linolenic acid (56.94–58.02%), with a total polyunsaturated fatty acid content of 75.49–76.47%, while saturated fatty acids and monounsaturated fatty acids constituted 10.53–11.11% and 13.00–13.52% of the total fatty acids, respectively.

Keywords: linolenic acid; *Perilla frutescens*; tocopherol

Introduction

Perilla frutescens (L) Britton is an oil crop extensively cultivated in East and Southeast Asian countries, such as China, South Korea, and Thailand, especially in the northern part of Thailand. Perilla is widely used as a food and traditional medicinal product. Perilla seeds contain 35–40% of crude fat (Asif, 2011). Recently, perilla oil has received more attention as a healthy food because it is a rich source of omega-3 fatty acids. It is regarded to have the highest omega-3 content among vegetable oils (Asif, 2011). In addition, omega-6 and omega-9 fatty acids are also present. Consequently, perilla oil is rich in polyunsaturated fatty acids (PUFAs), which have numerous health benefits for humans. Many studies exist on the relationship between omega-3 and the prevention or reduction of the risk of coronary heart disease (Cundiff *et al.*, 2007), cancer (Lu *et al.*, 2022), fatty liver disease, and metabolic syndrome (Jang and Park, 2020). It is well known that unsaturated fatty acids properly regulate blood lipid profile (Chopra and Sambaiah, 2009), improve insulin levels (Song *et al.*, 2020), anti-inflammatory and antioxidant properties (Oppedisano *et al.*, 2020), which help to reduce the risk of many chronic diseases. In addition, perilla oil contains phenolic compounds, such as rosmarinic acid and luteolin, which have antioxidant properties (Asif, 2011).

Oil extraction from oil seeds is commonly performed by mechanical pressing and solvent extraction. For mechanical pressing, oil seeds are roasted before pressing to increase oil yield. However, the heating method increases oxidative instability and degrades some bioactive compounds (Jung *et al.*, 2012). Hexane is generally used for solvent extraction. Achieving oil yield of up to 95% is possible, but it has some disadvantages regarding environmental hazards and might have solvent residues in products (Lavenburg *et al.*, 2021).

The supercritical fluid extraction method is an alternative method that is highly effective in extracting vegetable oils because of its high penetration and diffusion capabilities (Zhang *et al.*, 2018). Carbon dioxide (CO_2) is the most preferred supercritical solvent because of its low critical point, nontoxicity, simple separation, and ability to be removed entirely from the extract (Özkal, 2009). Studies exist on supercritical fluid extraction in seeds, such as flaxseeds (Özkal, 2009), perilla seeds (Jung *et al.*, 2012), hazelnuts (Özkal *et al.*, 2005), and sunflower seeds (Salgın *et al.*, 2006). It was discovered that oil extracted with supercritical fluids had a higher yield compared to conventional extraction methods.

Previous studies have shown that extraction with supercritical carbon dioxide (SC-CO_2) is superior to traditional methods, such as pressing and solvent extraction, in terms of extraction efficiency, selectivity, and preservation of bioactive compounds. Hao *et al.* (2021) reported that perilla seed oil extracted using SC-CO_2 exhibits strong antibacterial and antioxidant activities, with high polyphenol and flavonoid contents, and better storage stability, compared to pressing and solvent extraction. Yang *et al.* (2017) found that SC-CO_2 is an effective method for extracting oil from perilla seeds and can help preserve essential compounds, especially omega-3 fatty acids, with the extracted oil containing up to 76.18% α-linolenic acid (ALA). Fuhua *et al.* (2016) reported that the optimal conditions for extracting linolenic acid included a temperature of 40°C for 120 min, with a CO_2 flow rate of 20 L/h and a pressure of 25 MPa, resulting in a high extraction rate of 88.09%.

However, SC-CO_2 extraction has limitations in terms of high operating costs, which is a major obstacle to the adoption of this technique at industrial level. Therefore, optimizing the extraction process is essential to reduce production costs and ensure long-term investment viability. This study aimed to investigate the optimum conditions of perilla seed oil extraction using the supercritical fluid extraction method. Response surface methodology based on central composite design was used to obtain high oil yield, total phenolic content (TPC), and antioxidant activity. The physicochemical properties, fatty acid composition, and color of the extracted oil were evaluated as well.

Materials and Methods

Plant material and chemicals

Perilla seed samples were collected from Mae Fah Luang district, Chiang Rai, Thailand, in December 2022. Seeds were crunched to a powder form using a kitchen-type blender (Phillips, The Netherlands) to pass through a 1 × 1-mm sieve. The moisture content of perilla seed powder was determined to be 3–4%.

A standard α-, γ-, δ-tocopherol, standard solution of fatty acid methyl esters (FAMEs) (Supelco 37 Component FAME mix), gallic acid, 2,2′-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), and sodium carbonate (Na_2CO_3) were purchased from Sigma-Aldrich, USA. Methanol, ethanol, potassium hydroxide, sodium hydroxide, glacial acetic acid, chloroform, hexane, cyclohexane, hydrochloric acid, sodium thiosulfate, sodium chloride, Wijs solution, and phenolphthalein were purchased from RCI Labscan Ltd., Thailand. Boron trifluoride (BF_3) in methanol (20% w/v) and Folin–Ciocalteu reagent were purchased from Merck, Germany. Potassium persulfate was purchased from Panreac, Spain. Soluble starch was purchased from Ajax Finechem, Australia. Potassium iodide was purchased from Qrec, New Zealand.

Oil extraction

In all, 250 g of perilla seeds were extracted using SC-CO_2 extraction (Figures 1 and 2). The extraction was conducted at three different pressures (200, 225, and 250 bar) and temperatures (40°C, 50°C, and 60°C) for 2 h. During the experiment, the flow rate was controlled at 118.65–146.90 L/h. The extracted crude oil was collected in a sampling bottle. Each experiment was performed in triplicate. The extracted crude oil was centrifuged and filtered to remove waxes and impurities. Oil yield (%) was calculated using the following equation:

$$\text{Oil yield } (V) = \frac{W_o}{W_i} \times 100, \tag{1}$$

where W_o is the weight of the extracted oil (g), and W_i is the weight of dried perilla seeds used in extraction (g).

Experimental design

Response surface methodology (RSM) based on a central composite design (CCD) was used to determine the optimal conditions for perilla seed oil extraction. The uncoded and coded values of independent variables are given in Table 1.

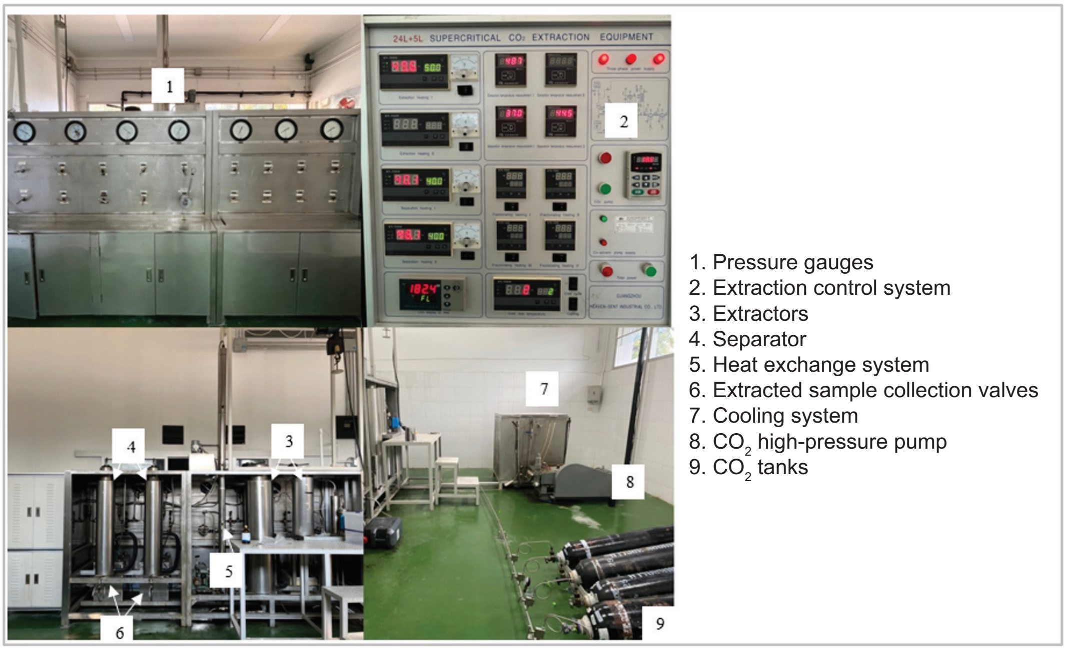

Figure 1. **Supercritical carbon dioxide (SC-CO$_2$) extraction machine and equipment.**

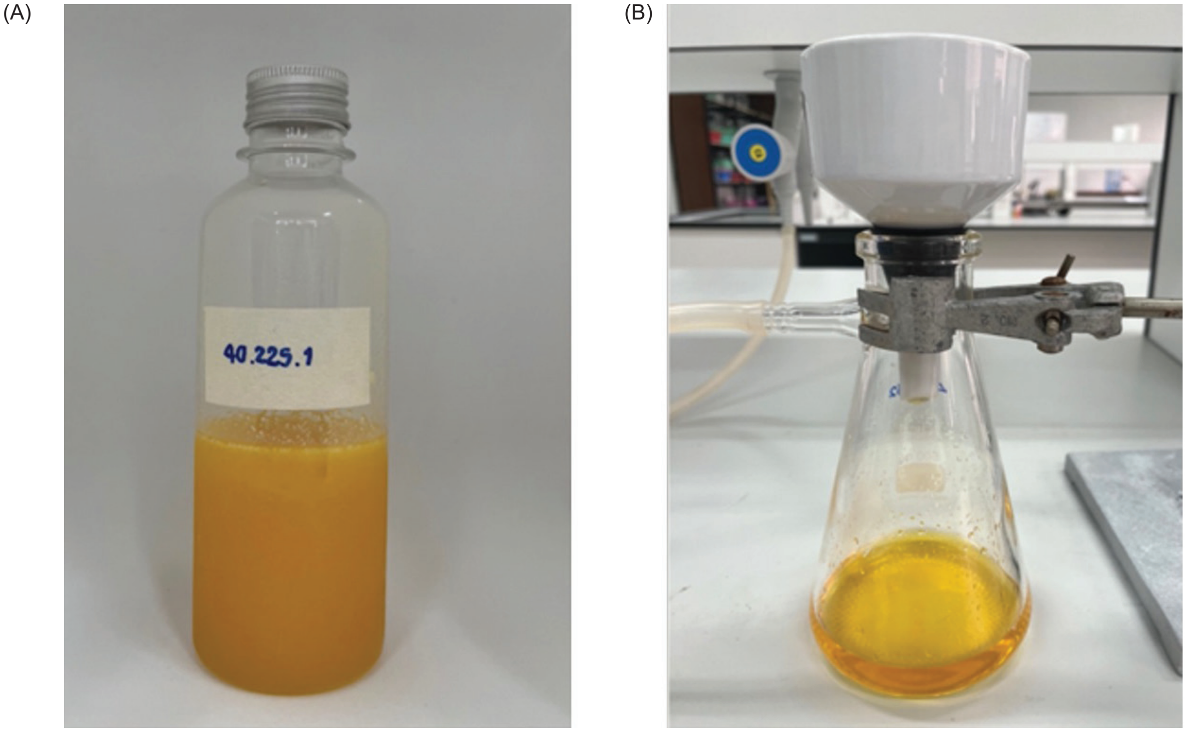

Figure 2. **Perilla seed oil extracted with supercritical CO$_2$ extraction: (A) crude perilla seed oil; and (B) perilla seed oil after centrifugation and filtration.**

Table 1. Coded and uncoded levels of independent variables for response surface methodology (RSM).

Treatment	Coded independent variables		Uncoded independent variables	
	Temperature (X_1, °C)	Pressure (X_2, bar)	Temperature (X_1, °C)	Pressure (X_2, bar)
1	–1	–1	40	200
2	1	–1	60	200
3	–1	1	40	250
4	1	1	60	250
5	–1	0	40	225
6	1	0	60	225
7	0	–1	50	200
8	0	1	50	250
9	0	0	50	225
10	0	0	50	225
11	0	0	50	225

The relationship between response values and independent variables was fitted with a second-order polynomial model according to Equation 2

$$Y = \beta_0 + \sum\beta_i X_i + \sum\beta_{ii} X_i^2 + \sum\beta_{ij} X_i X_j, \qquad (2)$$

where Y is the response variable, X_i and X_j are independent variables affecting the response, and β_0, β_i, β_{ii}, and β_{ij} are regression coefficients for the intercept (the constant), and linear, quadratic, and interaction terms. The experimental design, three-dimensional (3D) response surface, and desirability functions to determine optimum extraction conditions were constructed and analyzed using the Minitab software version 16.00.

Acid value, peroxide value, iodine value, and saponification value

Acid value (AV; mg of KOH/g of oil), peroxide value (PV; milliequivalent of oxygen/kg of oil [mEq/kg]), iodine value (IV; g of I_2/100 g of oil), and saponification value (SV; mg of KOH/g of oil) were determined according to the AOAC methods 940.28, 965.33, 920.159, and 920.160, respectively (Association of Official Analytical Chemists [AOAC], 2000).

Methanolic extract preparation

In all, 5 g of oil sample was extracted for three times with 15 mL of methanol by vortexing for 5 min. The solution was centrifuged at 5,000 rpm for 10 min. Supernatant was collected after centrifugation. The solvent was removed using a rotary evaporator, and the final volume was adjusted to 5 mL.

Total phenolic content

The TPC was determined according to the method described by Ahmed *et al.* (2019). Briefly, 0.5 mL of methanolic extract was mixed with 2.5 mL of 10% Folin–Ciocalteu reagent and incubated at room temperature for 5 min. After that, 2 mL of 7.5% Na_2CO_3 was added and left to stand for 90 min at room temperature. The absorbance was measured at 760 nm. TPC was calculated using a gallic acid standard curve and expressed as mg GAE/100 g of sample.

DPPH

The DPPH radical scavenging activity of oil was measured using the method described by Semiz *et al.* (2018). Briefly, 1 mL of methanolic extract was mixed with 1 mL of 0.06-mM DPPH. The mixture was kept in the dark for 30 min. Absorbance was obtained at 517 nm. The radical scavenging activity was expressed as a percentage using the following equation:

$$\text{Inhibition }(\%) = \frac{\text{Ab}_{\text{control}} - \text{Ab}_{\text{sample}}}{\text{Ab}_{\text{control}}} \times 100, \qquad (2)$$

where $\text{Ab}_{\text{sample}}$ is absorbance of the sample and $\text{Ab}_{\text{control}}$ is absorbance of the control.

ABTS

The ABTS radical scavenging activity was determined according to the method described by Gokbulut *et al.* (2013). A stock solution of ABTS+ was prepared by mixing 7-mM ABTS solution with 2.45-mM potassium persulfate (1:1) and allowed to stand in the dark at room temperature for 16 h. The ABTS+ stock solution was diluted with ethanol to obtain an absorbance value of 0.7 ± 0.02 at 734 nm. After that, 0.1 mL of methanolic extract was mixed with 1.9 mL of ABTS solution. Absorbance was measured after 8 min at 734 nm. The radical scavenging activity was expressed as a percentage according to Equation 2.

Tocopherol

The tocopherol content was determined according to the method described by Majid *et al.* (2019a). Tocopherols were extracted using methanol, and filtered through a 0.45-μm filter. Briefly, 10 μL of the sample was directly injected into a Chromaster series HPLC system (Hitachi, Japan) using a Pinnacle II C18 column (5 μm, 4.6 × 150 mm). The effluent was monitored with ultraviolet (UV) radiation at 292 nm. The mobile phase was methanol/water (98:2, v/v). The flow rate was 1 mL/min, and the run time was 20 min.

Color measurement

The color of perilla seed oil was measured using a Hunter Lab ColorQuest XE colorimeter (Hunter Associates Laboratory Inc., Reston, VA, USA) and expressed as L*, a*, and b* values in the CIELAB color space. L* represents lightness (0 for black and 100 for white), a* represents the red–green axis (positive for red and negative for green), and b* represents the yellow–blue axis (positive for yellow and negative for blue).

Fatty acid composition

Fatty acid methyl esters of perilla oil samples were prepared using FAMEs according to the method described by Morrison and Smith (1964). Briefly, 1 μL of FAMEs was injected into a Gas Chromatography (GC; Bruker, Scion 436-GC, Germany) using an RT-2560® column (bis cyanopropyl polysilozane) 100-m, 0.25-mm ID, 0.2-μm df (Restek®, USA), and a flame ionization detector (FID). Nitrogen was used as a carrier gas with a flow rate of 1 mL/min. The injector temperature was 225°C, and the detector temperature was 250°C.

Statistical analysis

All experiments were conducted in triplicate. The data were analyzed and compared using Analysis of Variance with Duncan's multiple range test at a confidence level of 95% using SPSS Statistic 17.0.

Results and Discussion

Oil yield

The yield percentage of oil ranged from 20.55% to 39.41%, and oil extracted at 60°C under 250 bar had the highest oil yield (Table 2). Based on Figure 3A, the oil yield increased with increasing pressure from 200 bar to 250 bar because the increase in pressure leads to a higher density of CO_2, enhancing oil solubility in CO_2 (Salgin and Salgın, 2006). The increasing temperature from 40°C to 50°C resulted in lower oil yield because an increase in temperature decreased the density of CO_2 and oil solubility (Döker et al., 2010). However, the oil yield increased when the temperature increased from 50°C to 60°C. This was because increasing temperature decreased the density of CO_2 and increased vapor pressure of the substance and the mass transfer (Wei *et al.*, 2021).

Various studies demonstrated that the extraction pressure is a significant parameter affecting the yield of extracted rape seed oil (Cvjetko Bubalo *et al.*, 2012), mucuna seeds meal oil (Garcia *et al.*, 2015), *Portulaca oleracea* seed oil (Sodeifian *et al.*, 2018), palm kernel oil (Sarker *et al.*, 2007), and argan oil (Taribak *et al.*, 2013). Increasing the extraction pressure enhanced the oil yield. Jiao *et al.* (2021) extracted oil from seeds and leaves using critical CO_2 extraction at 40–55°C at a pressure of 15–30 MPa. The yield increased as the extraction pressure increased from 15 MPa to 30 MPa. Increasing temperature from 40°C to 50°C increased the yield. However, the yield decreased when the temperature increased to 55°C. Scapin *et al.* (2017) extracted perilla seed oil with compressed CO_2 at 20–60°C and a pressure of 10–25 MPa.

Table 2. Percentage of oil yield.

Temperature (°C)	Pressure (bar)	Oil yield (%)
40	200	26.26 ± 1.82^{f}
60	200	31.73 ± 0.71^{e}
40	250	38.31 ± 0.44^{a,b}
60	250	39.41 ± 1.03^{a}
40	225	35.68 ± 3.49^{b,c,d}
60	225	37.97 ± 1.62^{a,b,c}
50	200	20.55 ± 2.62^{g}
50	250	36.12 ± 1.03^{a,b,c,d}
50	225	31.73 ± 1.32^{e}
50	225	33.82 ± 1.76^{c,d,e}
50	225	31.92 ± 2.71^{d,e}

Note. Data are expressed as mean values ± standard deviation (SD). Different superscripted letters in each column indicate significant differences at $P \leq 0.05$.

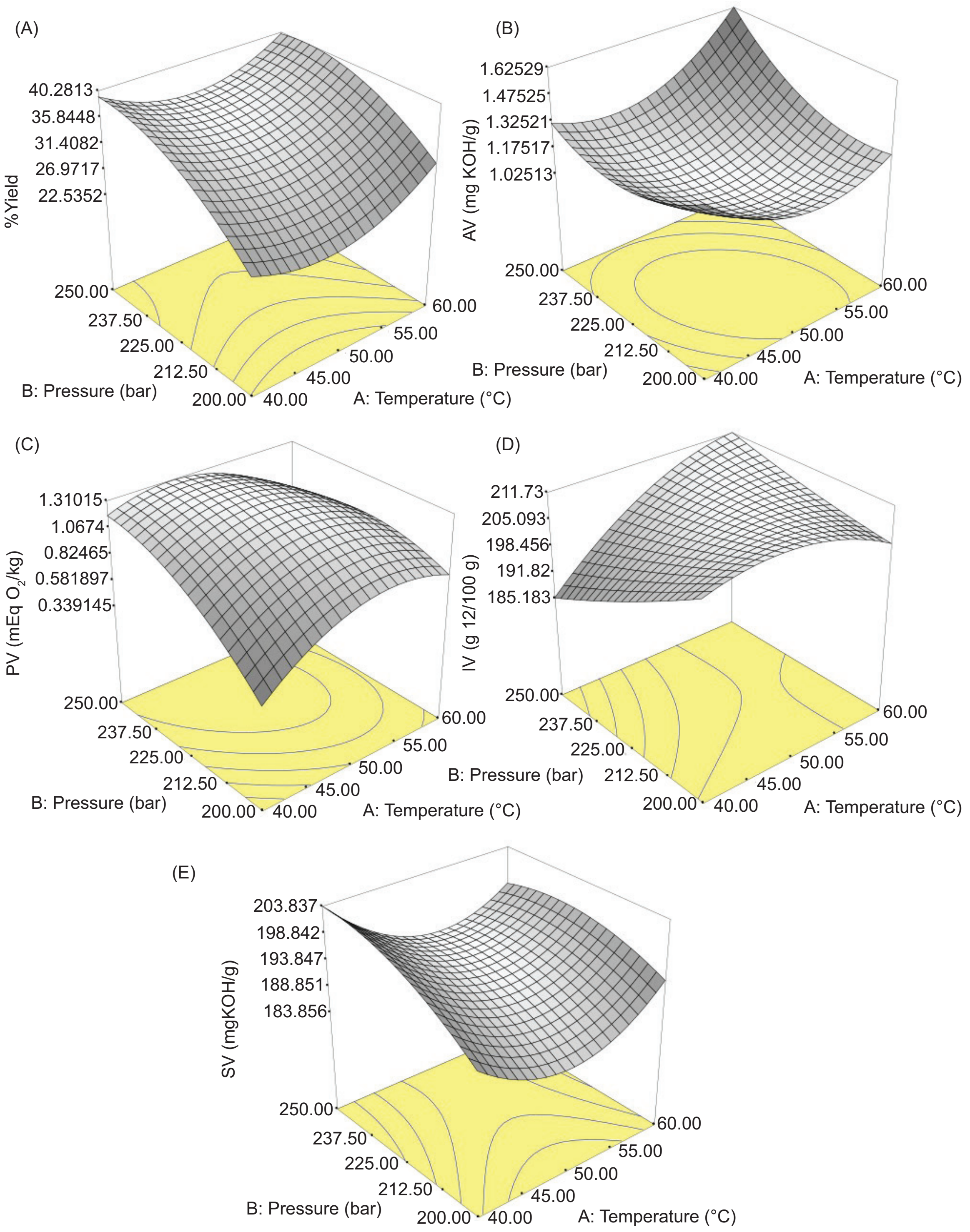

Figure 3. Response surface plot of (A) oil yield (%), (B) acid value (AV), (C) peroxide value (PV), (D) iodine value (IV), and (E) saponification value (SV).

It was found that increasing pressure at a constant temperature resulted in an increased oil yield. The highest yield (31.80%) was obtained at 20°C under a pressure of 25 MPa.

Previous studies demonstrated that the yield of perilla oil is approximately 35–42%, depending on the extraction method. Zhao *et al.* (2021) extracted perilla oil using cold press extraction (with 34.40% yield), solvent extraction (39.61% yield), aqueous enzymatic extraction (31.28% yield), and ultrasound-assisted hexane extraction (36.30% yield). Jung *et al.* (2012) reported that the yield of perilla oil extracted using

Table 3. Quadratic response surface models for oil yield (%), acid value (AV), peroxide value (PV), iodine value (IV), saponification value (SV), total phenolic content (TPC), DPPH (IC_{50}), ABTS (IC_{50}), and total tocopherol.

Response	Quadratic polynomial model	Adj. R^2	*P*-value
Oil yield (%)	$= -234.9366 + 2.9662 \times \text{Pressure} + 0.05 \times \text{Temp}^2 - 0.0056 \times \text{Pressure}^2$	0.9061	0.00
AV	$= 22.7231 + 0.002 \times \text{Temp}^2 + 0.0002 \times \text{Pressure}^2 + 0.0005 \times \text{Temp} \times \text{Pressure}$	0.8054	0.01
PV	$= -31.8401 + 0.1812 \times \text{Pressure} - 0.0028 \times \text{Temp}^2 - 0.0008 \times \text{Temp} \times \text{Pressure}$	0.8433	0.01
IV	$= 508.451 - 1.4642 \times \text{Temp} - 2.3393 \times \text{Pressure} - 0.0526 \times \text{Temp}^2 + 0.0322 \times \text{Temp} \times \text{Pressure}$	0.8505	0.01
SV	$= 8.8435 + 2.8802 \times \text{Pressure} + 0.0807 \times \text{Temp}^2 - 0.0051 \times \text{Pressure}^2$	0.9042	0.001
TPC	$= 199.6972 + 0.0041 \times \text{Pressure}^2$	0.7914	0.02
DPPH (IC_{50})	$= -1.7575 + 0.0715 \times \text{Pressure}$	0.7888	0.02
ABTS (IC_{50})	$= 537.5915 - 2.3547 \times \text{Pressure} + 0.1038 \times \text{Temp}^2$	0.7700	0.02
Total tocopherol	$= 3288.481 - 16.9898 \times \text{Pressure}$	0.7700	0.02

Table 4. Acid value (AV), peroxide value (PV), iodine value (IV), and saponification value (SV).

Temp (°C)	Pressure (bar)	Acid value (mg KOH/g oil)	Peroxide value (mEq O_2/kg Oil)	iodine value (g I_2/100 g)	Saponification value (mg KOH/g oil)
40	200	1.35 ± 0.21^{b}	0.30 ± 0.10^{d}	$206.83 \pm 3.03^{b,c,d}$	$190.48 \pm 2.93^{d,e}$
60	200	$1.23 \pm 0.17^{b,c}$	0.79 ± 0.10^{c}	$201.04 \pm 1.40^{e,f}$	$193.26 \pm 2.76^{c,d}$
40	250	1.27 ± 0.06^{b}	1.21 ± 0.15^{a}	184.22 ± 3.33^{g}	203.39 ± 1.08^{a}
60	250	1.64 ± 0.16^{a}	$0.89 \pm 0.13^{b,c}$	$210.63 \pm 3.34^{a,b}$	197.48 ± 1.45^{b}
40	225	1.28 ± 0.17^{b}	$0.94 \pm 0.13^{b,c}$	198.11 ± 1.51^{f}	201.91 ± 2.29^{a}
60	225	$1.24 \pm 0.19^{b,c}$	0.83 ± 0.20^{c}	$208.83 \pm 2.13^{a,b,c}$	$196.15 \pm 1.21^{b,c}$
50	200	$1.12 \pm 0.13^{b,c}$	0.85 ± 0.13^{c}	213.32 ± 1.91^{a}	183.97 ± 1.28^{f}
50	250	1.30 ± 0.21^{b}	$1.12 \pm 0.08^{a,b}$	$205.78 \pm 0.95^{c,d}$	$191.60 \pm 0.96^{d,e}$
50	225	0.97 ± 0.09^{c}	1.29 ± 0.20^{a}	$206.31 \pm 3.51^{b,c,d}$	$191.18 \pm 2.17^{d,e}$
50	225	$1.14 \pm 0.13^{b,c}$	1.19 ± 0.10^{a}	$205.47 \pm 2.09^{c,d,e}$	$193.42 \pm 2.34^{c,d}$
50	225	0.95 ± 0.11^{c}	1.34 ± 0.10^{a}	$202.38 \pm 2.54^{d,e,f}$	189.23 ± 2.07^{e}

Data are expressed as mean values ± standard deviation (SD). Different superscripted letters in each column indicate significant differences at $P \leq 0.05$.

SC-CO_2 at 50°C under 300-, 360-, and 420-bar pressure was 29.14%, 35.88%, and 39.56%, respectively. The yield of oil extracted at 50°C and 420 bar was not significantly different from that of hexane extraction, which yielded 41.76%, while mechanical pressing resulted in the lowest yield of 31.74%. This indicates that SC-CO_2 extraction is as effective as solvent extraction. Moreover, SC-CO_2 is considered a safe and environment-friendly technique.

Model fitting for yield (%), physicochemical properties, bioactive compounds, and antioxidant activity of extracted oil

In Table 3, the adjusted R^2 values of the quadratic models are 0.9061, 0.8054, 0.8433, 0.8505, 0.9042, 0.7914, 0.7888, 0.7700, and 0.770 for oil yield (%), AV, PV, IV, SV, TPC, DPPH (IC_{50}), ABTS (IC_{50}), and total tocopherol, indicating a good fit for most models. In spite of slight variations, all models were statistically significant ($P \leq 0.05$). This suggests that the models were sufficient in representing the relationship between factors and responses and predicting of responses.

Acid value, peroxide value, saponification value, and iodine value

Acid value measures the amount of free fatty acids in a sample. Higher AV indicates the presence of oxidation or hydrolysis that causes rancidity and reduces the quality of oil. In Table 4, AV ranges from 0.95 to 1.64 mg KOH/g oil. Oil extracted at a temperature of 50°C and a pressure of 225 bar had the lowest AV. According to Figure 3B, AV increases with pressure at 60°C because higher temperatures can accelerate oxidation in oil. In addition, AV increased with temperature at a pressure

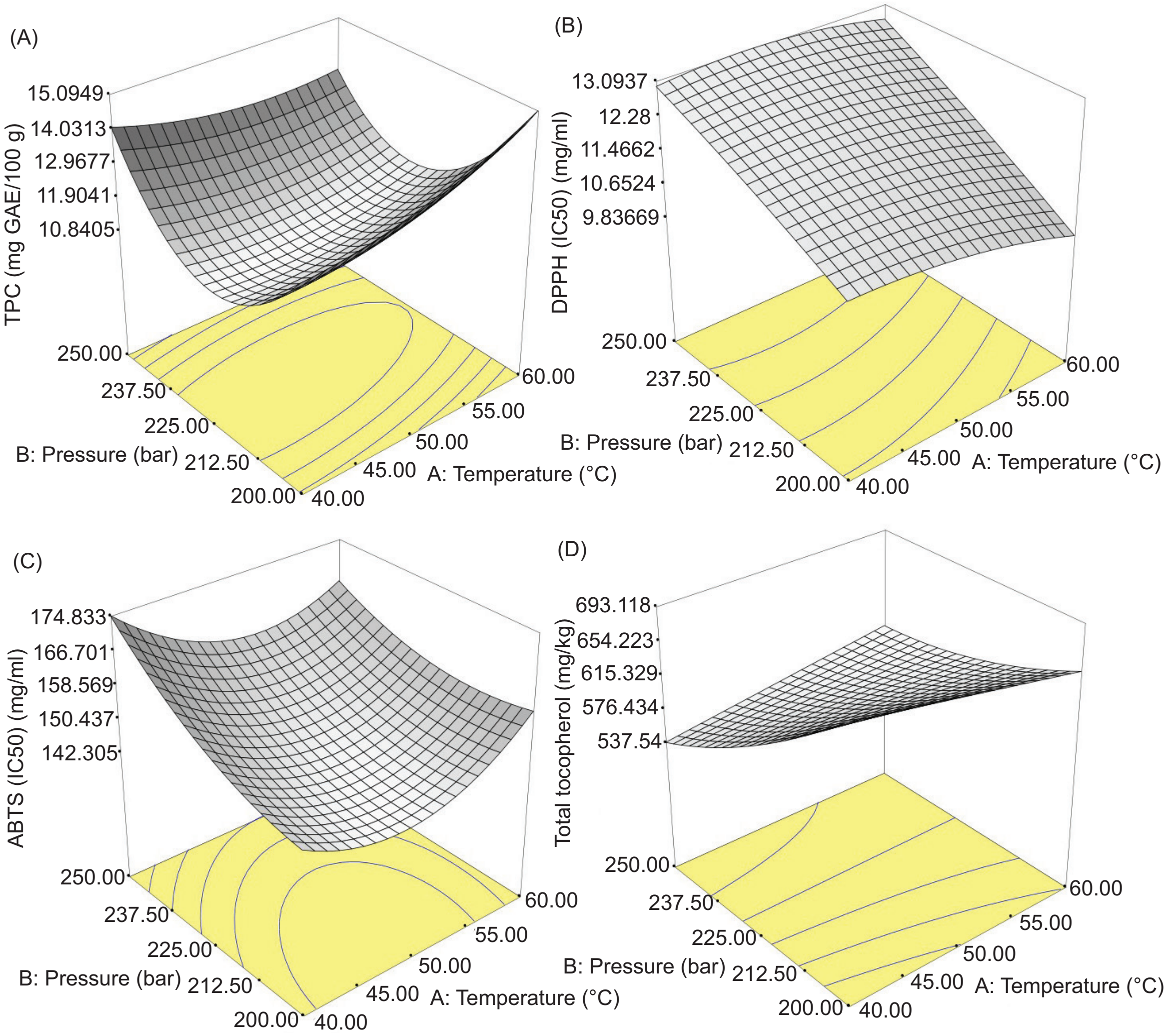

Figure 4. Response surface plots for (A) total phenolic content (TPC), (B) DPPH (IC_{50}), (C) ABTS (IC_{50}), and (D) total tocopherol.

of 250 bar. Increased pressure increased free fatty acid solubility (Muangrat and Pongsirikul, 2019), leading to a higher extraction of free fatty acids. According to Codex Alimentarius, oils are still acceptable with AV < 4 mg KOH/g oil. The AV of SC-CO_2 extraction was lower than the acceptable value, indicating a high oxidation stability of the oil.

Peroxide value indicates the oxidation of oils that causes rancidity and adversely affects the quality of oils. Higher PV suggests a higher level of oxidation and potential rancidity. In Table 4, PV ranges from 0.30 to 1.34 mEq O_2/kg of oil. At an extraction temperature of 40°C and an extraction pressure of 200 bar, oil had the lowest PV. Figure 3C demonstrates that PV increased with increasing extraction temperature from 40°C to 50°C. However, PV decreased as temperature increased from 50°C to 60°C because of the relatively high antioxidant and phenolic compound content at 60°C. On the other hand, PV increased with increasing extraction pressure. Muangrat and Jirarattanarangsri (2020) reported that PV of Assam tea seed oil increased when extraction pressure was more than 200 bar because higher pressure increased lipid oxidation. According Codex Alimentarius, oil is acceptable at PV < 15 mEq/kg of oil. Factors affecting PV include fatty acid composition and compounds with antioxidant properties, such as polyphenols and tocopherols (Sodeifian *et al.*, 2018; Tabee *et al.*, 2008). Since the unsaturated bonds in oil are prone to oxidation, the presence of substances with natural antioxidant properties slows down oxidation.

Iodine value is the amount of iodine in grams that reacts with the double bonds of unsaturated fatty acids in 100 g of oil, indicating a degree of unsaturation. A high IV indicates that oil contains a high content of unsaturated fatty acids. In Table 4, IV ranges from 184.22 to 213.32 g I_2/100 g of oil. The oil extracted at a temperature of 50°C and a pressure of 200 bar had the highest IV. According to Figure 3D, IV increased as the temperature increased from 50°C to 60°C because increasing temperature enhances the extraction of fatty acids. Except for 60°C, when the pressure increased from 200 bar to 250 bar, the IV decreased because more saturated fatty acids (SFA) are extracted at a higher pressure. The result was consistent for the fatty acid composition measured by GC. The PUFA–SFA ratio decreased when the extraction pressure increased from 200 bar to 250 bar, resulting in a decrease in the unsaturation level of the oil. The IV obtained in this experiment indicated that perilla oil is a rich source of unsaturated fatty acids, which are essential and beneficial to health.

Saponification value indicates the average molecular weight of fatty acids in oils. Oils with high SV contain low molecular weight fatty acids. SV is used to evaluate the chain length of fatty acids, in particular fats and oils (Ivanova *et al.*, 2022). In Table 4, SV ranges from 183.97 to 203.39 mg KOH/g of oil. The oil extracted at a temperature of 40°C and a pressure of 250 bar had the highest SV. According to Figure 3E, SV decreased as the temperature increased from 40°C to 50°C. However, the SV increased when the temperature increased from 50°C to 60°C. The SV increased with increasing pressure because of a higher content of short-chain fatty acid and a lower content of long-chain fatty acid at a higher extraction pressure.

Previous studies reported different physicochemical properties of perilla oil. Joshi *et al.* (2015) extracted oil from perilla seeds grown in India using Soxhlet extraction with petroleum ether as a solvent, obtaining an AV of 1.59 mg KOH/g of oil and a SV of 185.3 mg KOH/g of oil. Zhao *et al.* (2021) reported that perilla oil from perilla seeds grown in China extracted by cold pressing and hexane extraction had a PV of 1.60 mEq O_2/kg of oil and 1.78 mEq O_2/kg of oil, an IV of 197.6 194.4 g I_2/100 g and 194.4 g I_2/100 g, and an SV of 189.6 mg KOH/g of oil and 185.8 mg KOH/g of oil, respectively. Sirilun *et al.* (2016) extracted perilla oil using hexane extraction and reported that the oil had an AV and PV of approximately 20 mgKOH/g of oil and 25 mEq O_2/kg of oil, respectively. Differences in the values were attributed to the factors such as cultivation location, growing and harvesting seasons, and extraction methods. It is observed that perilla oil extracted using SC-CO_2 has lower AV and PV, compared to other extraction methods, indicating higher chemical stability, which means less oxidation and degradation potential. Meanwhile, in most of the cases, IV and SVs were within a comparable range.

Lipid oxidation adversely affects oil's stability, shelf life, and human health. Oil's susceptibility to oxidation mainly depends on the composition of fatty acids (Tabee *et al.*, 2008). Since unsaturated bonds in an oil are prone to oxidation, presence of natural antioxidants, such as polyphenols and tocopherols, slows down the oxidation process, with the ability to delay or inhibit the initiation step or prevent the propagation step of the lipid oxidation process (Shahidi and Ambigaipalan, 2015).

Total phenolic content and antioxidant capacity of extracted oil

In Table 5, TPC of the extracted oil ranged from 10.40 to 14.54 mg of GAE/100 g of oil. The highest content of TPC was observed at a temperature of 60°C and a pressure of 200 bar. Increasing temperature from 40°C to 60°C increased TPC (as shown in Figure 4A) because vapor pressure and diffusion coefficients of compounds increased with increase in temperature (Adil *et al.*, 2008). Moreover, increasing temperature decreased the density of CO_2, leading to an increase in the mass transfer of phenolic compounds. However, under a pressure of 250 bar, TPC decreased with increasing temperature because phenolic compounds are sensitive to environmental conditions, and exposure to elevated temperatures and/or pressures can result in their degradation or alter their biological functions (M'hiri *et al.*, 2015).

The scavenging activity of the extracted oil was expressed with IC_{50}; the lower value indicates a higher antioxidant capacity. DPPH IC_{50} was 9.93–13.27 mg/mL of the oil sample (Table 5). Oil extracted at a temperature of 60°C and pressure of 200 bar had the highest DPPH antioxidant capacity. The lowest value of DPPH IC_{50} was obtained at an extraction temperature of 60°C because of the relatively high phenolic compound content. In Figure 4B, DPPH IC_{50} decreased when the temperature increased from 40°C to 60°C due to the increase in vapor pressure of the antioxidant caused by higher temperature (Zhao and Zhang, 2014). Moreover, the diffusion coefficient of CO_2 and the solubility of antioxidants also increased, resulting in the extraction of more antioxidants. On the other hand, DPPH IC_{50} increased with increasing pressure because of the increased compaction of samples at higher pressure (Priyadarsani *et al.*, 2021), leading to a decrease in mass transfer and diffusion capacity, resulting in the reduced extraction of phenolic compound.

The IC_{50} value of ABTS scavenging activity was 141.81–178.79 mg/mL of the oil sample. The highest ABTS antioxidant activity was observed at a temperature of 50°C and a pressure of 225 bar. In Figure 4C, ABTS IC_{50} decreased when the temperature increased from 40°C to 50°C because of the effect of higher temperature on vapor pressure and solubility of antioxidants. On the other hand, when the temperature increased from 50°C to 60°C, ABTS IC_{50} increased because antioxidants were

Table 5. Total phenolics content (TPC), DPPH, and ABTS.

Temperature (°C)	Pressure (bar)	TPC mg GAE/100 g	DPPH (IC$_{50}$) (mg/mL)	ABTS (IC$_{50}$) (mg/mL)
40	200	13.34 ± 0.09^{c}	10.70 ± 0.35^{e,f}	148.72 ± 3.93^{d,e}
60	200	14.54 ± 0.39^{a}	9.93 ± 0.53^{f}	153.12 ± 2.60^{c,d}
40	250	14.44 ± 0.47^{a}	12.74 ± 0.46^{a,b}	178.79 ± 3.06^{a}
60	250	12.93 ± 0.18^{c,d}	12.75 ± 0.45^{a,b}	165.49 ± 10.23^{b}
40	225	10.40 ± 0.25^{f}	12.37 ± 0.40^{b,c}	153.87 ± 3.90^{c,d}
60	225	12.68 ± 0.07^{d}	11.24 ± 0.48^{d,e}	158.02 ± 4.53^{b,c}
50	200	13.94 ± 0.18^{b}	10.65 ± 0.30^{e,f}	146.84 ± 1.96^{d,e}
50	250	13.42 ± 0.26^{c}	13.27 ± 0.34^{a}	154.20 ± 4.58^{c,d}
50	225	10.26 ± 0.51^{f}	11.83 ± 0.59^{c,d}	141.81 ± 5.35^{e}
50	225	11.15 ± 0.11^{e}	11.16 ± 0.34^{d,e}	147.60 ± 2.57^{d,e}
50	225	10.90 ± 0.22^{e}	12.51 ± 0.80^{a,b,c}	149.36 ± 3.05^{c,d,e}

Data are expressed as mean values ± standard deviation (SD). Different superscripted letters in each column indicate significant differences at $P \leq 0.05$.

Table 6. Contents of α-tocopherol, γ-tocopherol, δ-tocopherol, and total tocopherols.

Temperature (°C)	Pressure (bar)	α-Tocopherol (mg/kg oil)	γ-Tocopherol (mg/kg oil)	δ-Tocopherol (mg/kg oil)	Total tocopherols (mg/kg oil)
40	200	7.70 ± 2.76^{ns}	690.63 ± 45.82^{a}	9.57 ± 2.71^{a}	707.91 ± 49.70^{a}
60	200	7.53 ± 0.36^{ns}	636.98 ± 9.95^{b,c}	7.13 ± 0.49^{a,b}	651.64 ± 10.42^{b}
40	250	6.53 ± 1.09^{ns}	526.93 ± 11.23^{f}	6.55 ± 0.86^{b}	543.29 ± 13.74^{e}
60	250	6.19 ± 0.45^{ns}	565.85 ± 25.57^{e,f}	6.79 ± 0.46^{b}	578.83 ± 25.61^{c,d,e}
40	225	6.13 ± 1.13^{ns}	567.73 ± 13.68^{d,e,f}	6.75 ± 0.71^{b}	580.61 ± 12.10^{c,d,e}
60	225	5.90 ± 1.11^{ns}	567.35 ± 12.55^{d,e,f}	6.70 ± 1.48^{b}	579.94 ± 14.98^{c,d,e}
50	200	7.45 ± 1.21^{ns}	663.92 ± 43.95^{a,b}	9.15 ± 1.90^{a,b}	640.31 ± 54.09^{b}
50	250	5.32 ± 0.84^{ns}	536.57 ± 8.90^{e,f}	6.83 ± 0.86^{b}	548.72 ± 9.11^{d,e}
50	225	7.11 ± 1.96^{ns}	582.46 ± 10.66^{d,e}	6.99 ± 1.43^{b}	603.46 ± 15.53^{b,c,d}
50	225	6.36 ± 0.96^{ns}	541.95 ± 19.74^{e,f}	6.79 ± 1.07^{b}	596.64 ± 40.67^{b,c,d,e}
50	225	6.76 ± 0.73^{ns}	612.86 ± 32.20^{c,d}	7.85 ± 0.91^{a,b}	627.47 ± 32.62^{b,c}

Data are expressed as mean values ± standard deviation (SD). Different superscripted letters in each column indicate significant differences at $P \leq 0.05$; ns: no significant difference at $P \leq 0.05$.

Table 7. Color value.

Temperature (°C)	Pressure (bar)	L*	a*	b*
40	200	94.24 ± 0.11^{a}	–8.64 ± 0.28^{e}	72.72 ± 0.98^{e}
60	200	93.58 ± 0.33^{b}	–6.26 ± 0.52^{c,d}	77.93 ± 0.31^{d}
40	250	92.86 ± 0.05^{c,d}	–6.69 ± 0.46^{d}	88.80 ± 0.30^{b}
60	250	92.32 ± 0.06^{d}	–4.71 ± 0.08^{a}	88.01 ± 0.23^{b,c}
40	225	94.23 ± 0.28^{a}	–9.42 ± 0.41^{e}	86.83 ± 0.40^{c}
60	225	93.30 ± 0.01^{b,c}	–5.10 ± 0.04^{a,b}	88.62 ± 0.19^{b}
50	200	93.28 ± 0.21^{b,c}	–5.29 ± 0.17^{a,b}	88.57 ± 0.25^{b}
50	250	92.87 ± 0.49^{c,d}	–5.02 ± 0.32^{a,b}	90.86 ± 0.95^{a}
50	225	93.44 ± 0.22^{b,c}	–5.90 ± 0.81^{b,c,d}	88.92 ± 0.85^{b}
50	225	93.49 ± 0.20^{b}	–5.50 ± 0.17^{a,b,c}	88.65 ± 0.30^{b}
50	225	93.34 ± 0.25^{b,c}	–5.61 ± 0.06^{a,b,c}	89.03 ± 0.44^{b}

Data are expressed as mean values ± standard deviation (SD). Different superscripted letters in each column indicate significant differences at $P \leq 0.05$.

highly susceptible to degradation if exposed to various conditions, particularly elevated temperatures (Ling *et al.*, 2022); this resulted in a reduced antioxidant activity. Similar to DPPH, increasing pressure from 200 bar to 250 bar increased ABTS IC$_{50}$ because of the negative effect of pressure on solute vapor pressure and diffusion capacity of CO$_2$.

Tocopherol

Tocopherol is a natural antioxidant present in vegetable oil that prevents lipid oxidation (Tabee *et al.*, 2008). Content of tocopherol ranged from 552.78 mg/kg to 707.91 mg/kg in perilla seed oil, as shown in Table 6. The highest tocopherol content was observed at a temperature of 40°C and a pressure of 200 bar. The most abundant tocopherol in perilla oil was γ-tocopherol (690.63–526.93 mg/kg), followed by δ-tocopherol (6.55–9.57 mg/kg) and α-tocopherol (5.32–7.7 mg/kg). In Figure 4D, content of tocopherol decreased with increasing pressure because of the increased compaction of samples (Priyadarsani *et al.*, 2021) and density of CO$_2$, which reduced the ability of CO$_2$ to penetrate sample matrix, resulting in a lower diffusion ability and mass transfer. Similarly, Jung *et al.* (2012) reported that perilla oil extracted using the SC-CO$_2$ at a temperature of 50°C and pressures of 300-, 360-, and 420-bar had tocopherol contents of 503.26, 444.83, and 420.16 mg/kg, respectively. In addition, Jung *et al.* (2012) indicated that perilla oil extracted by solvent extraction and mechanical pressing had respective tocopherol contents of 411.46 mg/kg and 400.71 mg/kg.

Previous studies reported total phenolic content and antioxidant capacity of perilla oil extracted by different methods. Lee *et al.* (2021) extracted perilla oil by hydraulic pressing and superheated steam treatment prior to hydraulic pressing and found that the respective total phenolic content was 4.32 mg of GAE/100 g and 12.8 mg of GAE/100 g of oil, and the respective DPPH radical scavenging activity was 6.35% and 32%. Pan *et al.* (2020) extracted perilla seed oil using hexane as a solvent, followed by a multi-step refining process. The total phenolic compounds were 7.91–9.40 mg of GAE/100 g of oil and the DPPH values were 1.78–1.87 MmolTEAC/g. Hao *et al.* (2021) extracted perilla oil using different methods, such as SC-CO$_2$ extraction, pressing, petroleum ether extraction, and using commercial perilla oil. The results showed total phenolic compounds as follows: 130.40 mg of GAE/100 g of oil (SC-CO$_2$ extraction), 87.70 mg of GAE/100 g of oil (by pressing), 28.70 mg of GAE/100 g of oil (using petroleum ether extraction), and 7.57 mg of GAE/100 g of oil (with commercial perilla oil). In addition, perilla oil extracted using SC-CO$_2$ also had the highest antioxidant activity, with the DPPH EC$_{50}$ value of 7.01 mg/mL and the ABTS EC$_{50}$ value of 12.75 mg/mL.

Table 8. Fatty acid composition (g/100 g, %)

Temperature (°C)	Pressure (bar)	Palmitic acid (C16:0)	Palmitoleic acid (C16:1)	Stearic acid (C18:0)	Oleic acid (C18:1n9c)	Linoleic acid (C18:2n6c)	Arachidic acid (C20:0)	Gamma-linolenicacid (C18:3n6)	Alpha-linolenicacid (C18:3n3)
40	200	7.06 ± 0.05^{e}	0.13 ± 0.01^{a,b,c}	3.28 ± 0.14^{d}	12.86 ± 0.29^{c}	18.15 ± 0.11^{d,e}	0.18 ± 0.01^{b}	0.20 ± 0.00^{ns}	58.02 ± 0.63^{a}
60	200	7.32 ± 0.12^{b}	0.15 ± 0.05^{a,b}	3.30 ± 0.07^{d}	12.89 ± 0.07^{c}	18.20 ± 0.01^{c,d,e}	0.19 ± 0.02^{a,b}	0.21 ± 0.02^{ns}	57.74 ± 0.09^{a}
40	250	7.18 ± 0.02^{c,d}	0.11 ± 0.03^{b,c}	3.51 ± 0.01^{a,b}	13.36 ± 0.13^{a}	18.37 ± 0.04^{a}	0.18 ± 0.01^{b}	0.18 ± 0.02^{ns}	57.10 ± 0.12^{d,e,f}
60	250	7.17 ± 0.04^{c,d,e}	0.15 ± 0.02^{a,b}	3.55 ± 0.03^{a}	13.38 ± 0.04^{a}	18.35 ± 0.05^{a,b}	0.22 ± 0.02^{a}	0.23 ± 0.02^{ns}	56.96 ± 0.04^{f}
40	225	7.23 ± 0.03^{b,c,d}	0.14 ± 0.00^{a,b,c}	3.51 ± 0.03^{a,b}	13.30 ± 0.03^{a,b}	18.35 ± 0.03^{a,b}	0.18 ± 0.00^{b}	0.18 ± 0.01^{ns}	57.12 ± 0.08^{d,e,f}
60	225	7.15 ± 0.11^{d,e}	0.11 ± 0.01^{b,c}	3.50 ± 0.03^{a,b}	13.18 ± 0.04^{a,b}	18.37 ± 0.12^{a}	0.2 ± 0.00^{a,b}	0.22 ± 0.01^{ns}	57.28 ± 0.07^{d,e,f}
50	200	7.17 ± 0.03^{c,d,e}	0.09 ± 0.04^{c}	3.42 ± 0.06^{a,b,c}	13.12 ± 0.02^{a,b,c}	18.35 ± 0.02^{a,b}	0.18 ± 0.01^{a,b}	0.20 ± 0.01^{ns}	57.48 ± 0.10^{b,c,d}
50	250	7.17 ± 0.02^{c,d,e}	0.14 ± 0.02^{a,b}	3.49 ± 0·00^{a,b}	13.18 ± 0.02^{a,b}	18.25 ± 0.00^{a,b,c,d}	0.19 ± 0.04^{a,b}	0.20 ± 0.04^{ns}	57.38 ± 0.01^{c,d,e}
50	225	7.11 ± 0.03^{d,e}	0.15 ± 0.01^{a,b}	3.35 ± 0.05^{c,d}	13.03 ± 0.32^{b,c}	18.10 ± 0.12^{e}	0.18 ± 0.02^{b}	0.20 ± 0.03^{ns}	57.83 ± 0.19^{a,b}
50	225	7.52 ± 0.00^{a}	0.17 ± 0.03^{a}	3.44 ± 0.08^{a,b,c}	13.26 ± 0.06^{a,b}	18.22 ± 0.06^{b,c,d}	0.19 ± 0.01^{a,b}	0.54 ± 0.59^{ns}	56.94 ± 0.09^{f}
50	225	7.28 ± 0.09^{b,c}	0.18 ± 0.05^{a}	3.54 ± 0.04^{a,b}	13.31 ± 0.07^{a,b}	18.30 ± 0.04^{a,b,c}	0.21 ± 0.02^{a,b}	0.21 ± 0.04^{ns}	56.98 ± 0.12^{e,f}

Data are expressed as mean values ± standard deviation (SD). Different superscripted letters in each column indicate significant differences at $P \leq 0.05$; ns: no significant difference at $P \leq 0.05$.

Zhao *et al.* (2021) analyzed unsaponifiable matters by gas chromatography–mass spectroscopy (GC-MS) and found that perilla oil is a good source of phytosterols (0.39 g/100 g), squalene (275.4 mg/kg), and tocopherols (154.37 mg/kg). It is demonstrated that SC-CO_2 extraction is a highly efficient method for extracting phenolic compounds and other bioactive substances.

It is well known that tocopherols and phenolic compounds significantly influence antioxidant activity. Perilla oil is rich in phenolic compounds, such as rosmarinic acid, caffeic acid, and ferulic acid, as well as various flavonoids, such as apigenin, luteolin, and catechins (Bae *et al.*, 2020). The oil is also a source of tocopherols, especially γ-tocopherol, resulting in the excellent antioxidant properties of perilla oil. Phenolic and tocopherol compounds are also considered beneficial in preventing certain chronic diseases, such as cardiovascular disease and cancer (Pham-Huy *et al.*, 2008). SC-CO_2 can extract higher quantities of bioactive compounds that not only enhance the nutritional value of the oil, increase its market value, and provide health benefits but also improve oil's oxidative stability.

Color

The color values of perilla seed oil extracted by SC-CO_2 under various conditions are shown in Table 7. It was found that as the pressure increased, the L* value decreased, indicating that the oil became darker. A decreasing a* value indicated a green color, while an increasing a* value suggested that the oil became less green. A positive b* value indicated a yellow color, and an increasing b* value demonstrated that the oil became more yellow. Yellow color could result from the greater extraction of yellow pigments, such as carotenoids. Perilla seed oil consists of carotenoids, with lutein and β-carotene being the primary carotenoids (Cruz *et al.*, 2022). Carotenoids are the substances responsible for red, yellow, and orange colors in vegetable oils (Valencia *et al.*, 2024). Increased carotenoid content makes the oil more yellow and less green. Increasing the temperature caused L* value to decrease, indicating that the oil became darker. This could be due to the oxidation of oil at high temperatures, which caused the oil to darken. The a* value indicated a decrease in the green hue, and the b* value increased when the temperature increased from 40°C to 50°C, indicating an increase in yellow color. However, the b* value decreased when the temperature increased from 50°C to 60°C, probably because of the degradation of carotenoids, which are sensitive to heat (Zepka and Mercadante, 2009).

Fatty acid composition

The fatty acid composition of perilla oil is shown in Table 8. The most abundant fatty acid in perilla oil was α-linolenic acid (56.94–58.02%), followed by linoleic acid (18.1–18.37%), oleic acid (12.86–13.38%), palmitic acid (7.06–7.52%), and stearic acid (3.28–3.55%). The fatty acid composition of the extracted oil with each condition was slightly different. In Table 9, the highest amount of PUFA is extracted at a pressure of 200 bar and it decreased with increasing pressure. PUFA–SFA ratio is the most commonly used index to evaluate the nutritional value of food. A high PUFA (%) and PUFA–SFA ratio and a low ω-6–ω-3 ratio indicate that perilla oil has a high nutritional value.

Table 9. SFA (%), MUFA (%), PUFA (%), PUFA–SFA ratio, and ω-6–ω-3 ratio.

Temperature (°C)	Pressure (bar)	SFA (%)	MUFA (%)	PUFA (%)	PUFA:SFA	ω-6:ω-3
40	200	10.53 ± 0.20^{e}	13.00 ± 0.29^{e}	76.47 ± 0.49^{a}	7.26 ± 0.18^{a}	0.32 ± 0.01^{c}
60	200	$10.81 \pm 0.08^{c,d}$	$13.04 \pm 0.03^{d,e}$	$76.15 \pm 0.11^{a,b}$	$7.04 \pm 0.06^{b,c}$	$0.32 \pm 0.00^{a,b,c}$
40	250	$10.87 \pm 0.01^{b,c}$	$13.47 \pm 0.16^{a,b}$	$75.66 \pm 0.16^{c,d}$	$6.96 \pm 0.02^{c,d}$	$0.33 \pm 0.00^{a,b}$
60	250	$10.94 \pm 0.05^{a,b,c}$	13.52 ± 0.04^{a}	75.54 ± 0.03^{d}	$6.91 \pm 0.03^{c,d,e}$	0.33 ± 0.00^{a}
40	225	$10.92 \pm 0.04^{a,b,c}$	$13.44 \pm 0.03^{a,b,c}$	$75.64 \pm 0.06^{c,d}$	$6.93 \pm 0.03^{c,d,e}$	$0.32 \pm 0.00^{a,b,c}$
60	225	$10.84 \pm 0.11^{b,c}$	$13.3 \pm 0.04^{a,b,c,d}$	$75.86 \pm 0.10^{b,c,d}$	$7.00 \pm 0.08^{c,d}$	$0.32 \pm 0.00^{a,b}$
50	200	$10.77 \pm 0.07^{c,d}$	$13.2 \pm 0.02^{b,c,d,e}$	$76.03 \pm 0.09^{b,c}$	$7.06 \pm 0.05^{b,c}$	$0.32 \pm 0.00^{a,b,c}$
50	250	$10.84 \pm 0.06^{b,c}$	$13.32 \pm 0.00^{a,b,c}$	$75.83 \pm 0.05^{b,c,d}$	$6.99 \pm 0.04^{c,d}$	$0.32 \pm 0.00^{a,b,c}$
50	225	$10.64 \pm 0.01^{d,e}$	$13.19 \pm 0.32^{c,d,e}$	$76.17 \pm 0.33^{a,b}$	$7.16 \pm 0.04^{a,b}$	$0.32 \pm 0.00^{b,c}$
50	225	11.11 ± 0.15^{a}	$13.40 \pm 0.09^{a,b,c}$	75.49 ± 0.21^{d}	6.79 ± 0.11^{e}	0.33 ± 0.01^{a}
50	225	$11.02 \pm 0.13^{a,b}$	13.48 ± 0.03^{a}	75.49 ± 0.17^{d}	$6.85 \pm 0.10^{d,e}$	$0.32 \pm 0.00^{a,b,c}$

Data are expressed as mean values ± standard deviation (SD). Different superscripted letters in each column indicate significant differences at $P \leq 0.05$.

Similarly, Jung *et al.* (2012) reported that perilla oil extracted with SC-CO_2, pressing, and solvent extraction contained α-linolenic acid (61.69–62.63%), linoleic acid (14.03–14.13%), oleic acid (16.28–16.44%), palmitic acid (5.85–6.00%), and stearic acid (1.89–1.93%). In addition, different extraction methods had no significant effect on the composition of fatty acids. The results may vary slightly, possibly because of differences in species, planting locations, and seasons.

Optimization of supercritical CO_2 extraction

The optimization of perilla seed oil extraction by SC-CO_2 extraction was carried out to provide high oil yield and quality. The optimum conditions for oil extraction are shown in Figure 5. The optimum conditions were a temperature of 60°C under a pressure of 218 bar. Under this extraction condition, the respective predicted values of oil yield (%), AV, PV, IV, SV, TPC, DPPH (IC_{50}), ABTS (IC_{50}), and tocopherol were 37.00%, 1.22 mg KOH/g, 0.94 mEq O_2/kg, 205.00 g I_2/100 g, 196.90 mg KOH/g, 12.14 mg GAE/100 g, 10.93 mg/mL, 154.86 mg/mL, and 604.58 mg/kg.

The usage of SC-CO_2 extraction technology is influenced by many factors, such as increasingly strict environmental regulations to reduce hazardous chemicals, food safety norms, and the increasing demand for high-quality products (Amaral *et al.*, 2017). SC-CO_2 extraction eliminates the use of toxic chemicals, such as hexane, and is beneficial to the environment because no chemical residues are left in the product and it reduces environmental contamination. Moreover, CO_2 can be recycled and reused (Sharan and Madan, 2018). CO_2 is recognized as a safe option by regulatory authorities, including the Food and Drug Administration (FDA) and the European Food Safety Authority (EFSA) (Majid, *et al.*, 2019b), providing high-purity and high-quality products. SC-CO_2 extraction effectively preserves essential compounds such as omega-3 fatty acids and antioxidants, making it desirable for supplements and pharmaceutical industries requiring high product quality and safety. Moreover, with the increasing demand for natural and health-conscious products, the market for oils extracted through environment-friendly methods, such as SC-CO_2, is expanding, positioning SC-CO_2 extraction as a competitive method in the growing wellness and food markets.

However, this process is energy-intensive and has a high investment cost, especially the initial investment in equipment because of the use of high-pressure and complex equipment, compared to other extraction methods (Devittori *et al.*, 2000). Effective energy management, including CO_2 recycling, and optimizing extraction parameters could enhance the efficiency of the extraction process. In the long term, this approach reduces expenses and energy consumption while ensuring sustainability.

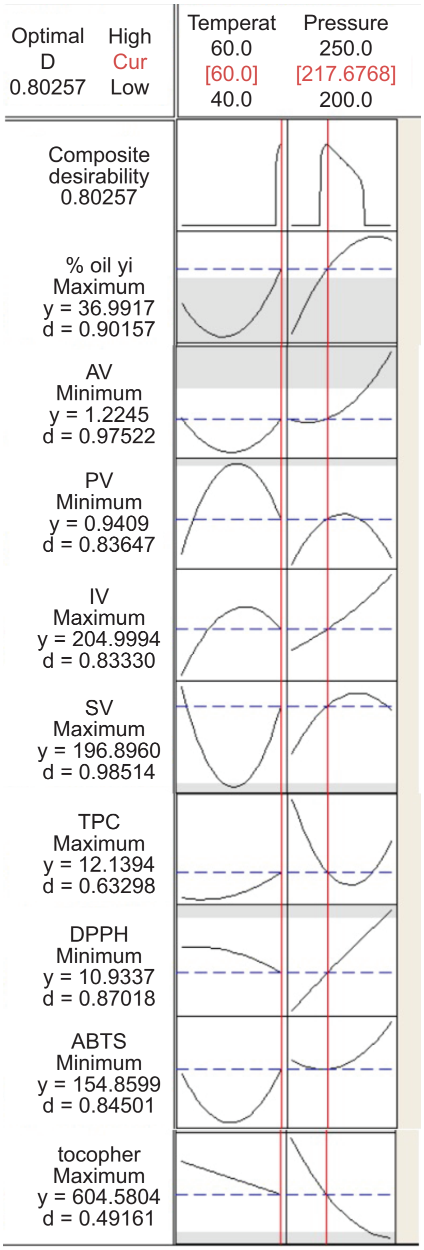

Figure 5. Analysis of extraction parameters affecting oil yield (%), acid value (AV), peroxide value (PV), iodine value (IV), saponification value (SV), total phenolic content (TPC), DPPH (IC_{50}), ABTS (IC_{50}), and tocopherol at the optimal conditions of SC-CO_2 extraction.

Conclusions

Optimum conditions of oil extraction from perilla seeds using SC-CO_2 extraction were investigated. The extraction was conducted at 200-, 225-, and 250-bar operating pressures and temperatures of 40°C, 50°C, and 60°C. The results showed that temperature and pressure were important factors affecting yield and physicochemical properties of oil. The increased pressure resulted in an increased yield; on the other hand, a decrease was observed in bioactive compounds, such as phenolic compounds, antioxidants, and tocopherol. Increase in temperature increased yield and bioactive compounds. However, increased temperature also increases AV and PV values. The optimum experimental extraction condition for high yield, total phenolic content, antioxidant activity, tocopherol, IV, and SV with low values of AV and PV was obtained at a temperature of 60°C with a pressure of 219 bar. It is suggested that perilla oil extracted using SC-CO_2 is suitable for use in the food and supplement industry.

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Author Contributions

All Authors contributed equally to this manuscript.

Data Availability Statement

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of Interest

None.

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