



Pilot-Scale Extraction of Cricket Oil (*Acheta domesticus*) Using Supercritical Carbon Dioxide

Dolaya Sadubsarn^{*1}, and Rattana Muangrat²

¹Department of Food Science and Technology, Faculty of Agro-industry, Chiang Mai University, Chiang Mai, Thailand

²Department of Food Process Engineering, Faculty of Agro-industry, Chiang Mai University, Chiang Mai, Thailand

^{*}Corresponding author, E-mail: dolaya.sds@gmail.com

Abstract

Edible insects are emerging as a novel food source as they are rich in nutrients. Although insect protein has been widely studied, research on insect oils is still limited. This study applied supercritical carbon dioxide (SC-CO₂) to extract oil from house crickets (*Acheta domesticus*). The experiment was designed using a Box-Behnken design (BBD) combined with the response surface method to optimize conditions for oil yield. The extraction was conducted at temperatures of 40–60°C, pressures of 175–225 bar, and extraction times of 1–5 h. The physicochemical properties of the oil, including acid value (AV), peroxide value (PV), iodine value (IV), saponification value (SV), and antioxidant activity, were analyzed. The result showed that higher pressure significantly increased oil yield ($p > 0.05$), while temperature provided a negative effect ($p > 0.05$). The highest experimental crude oil yield of 16.20% was obtained under optimal conditions of 60°C, 175 bar, and 5 h. Regarding physicochemical properties, the PV increased with temperature (40–50°C). In contrast, the IV decreased significantly with increasing temperature, while the SV exhibited a significant increase as the extraction temperature increased. Furthermore, the AV significantly increased as pressure decreased. Additionally, the oil showed significant antioxidant activity, as evidenced by ABTS and DPPH radical scavenging activities and total phenolic content.

Keywords: Supercritical carbon dioxide extraction, Cricket oil, Response surface methodology, Antioxidant activity, Edible insects, *Acheta domesticus*

1. Introduction

The global population continues to increase and is estimated to reach approximately 10 billion by the year 2050. This population growth leads to high demand for food; consequently, the new food source should be considered. Insects can be a potential novel food source, as they contain a high amount of protein as well as fat and essential minerals (Fornari et al., 2023). Over 110 countries worldwide have been reported consuming insects, particularly in Asian and African countries, and around 2100 insect species have been chosen for human consumption. In Thailand, it has been reported to consume more than 164 insect species, with the most popularly cultivated species being *Acheta domesticus* and *Gryllus bimaculatus* (Magara et al., 2021). *A. domesticus* (house cricket) is recognized as a potential food source of protein, fat, and vitamins, as well as essential minerals, and it has been reported that house crickets contain protein up to 76.19% dry weight and fat up to 43.9% dry weight (Pilco-Romero et al., 2023). Although insects have high potential as a food source, consumer acceptance remains a challenge, especially in European countries. To overcome this challenge, insects were transformed into powder, which can be used as a food ingredient (Megido et al., 2016). Beside the protein purification process, oil is a by-product and is often discarded. Therefore, extracting oil from insects is one way that can increase acceptance of insect consumption, as the process of oil extraction and purification will give oil that no longer resembles its insect source (Brogan et al., 2023).

As regards house crickets, the study by Brogan et al. (2023) revealed that house crickets contained the highest abundance of linoleic acid compared to locusts and silkworms. In addition, Pilco-Romero et al. (2023) also reported that house crickets contained sufficient amounts of linoleic, oleic, palmitic, and stearic acids, which might be an important source of fatty acids that have health benefits. Linoleic and oleic have beneficial effects in reducing the risk of cardiovascular disease (Pilco-Romero et al., 2023). Linoleic acid is

[416]



an essential fatty acid that the human body cannot synthesize and must be obtained through the diet (Mercola, & D'Adamo, 2023). Therefore, the oil extracted from house crickets should be studied and further developed at the industrial level to add value to cricket oil.

Traditional methods such as solvent-based extraction and mechanical pressing have been used for cricket oil extraction (Chaiyana et al., 2024; Laroche, et al., 2019). While mechanical extraction is known as a solvent-free and cost-effective method but it provides a low oil yield. Solvent-based extraction may leave harmful residues in the oil, thereby compromising its quality and safety, while mechanical pressing typically results in lower oil yield and does not effectively preserve sensitive bioactive compounds. In contrast, SC-CO₂ is a solvent-free method that offers higher efficiency and better preservation of the oil's bioactive properties (Purschke et al., 2017; Schoss, & Glavač, 2024). SC-CO₂ has been employed to extract oil from insects, including *T. molitor* L. larvae and black soldier fly larvae (Kim et al., 2019; Purschke et al., 2017). Furthermore, Purschke et al. (2017) studied the efficiency of SC-CO₂ extraction compared to hexane extraction in *Tenebrio molitor* L. larvae. The efficiency of oil extraction and oil composition were not significantly different between the two methods, where the SC-CO₂ can recover oil up to 95% with a high content of unsaturated fatty acids. Even though SC-CO₂ demonstrated a high potential in oil extraction, there are limited studies focused on optimizing the oil extraction process specifically in-house crickets. The limitation in existing research made it suitable for subjecting this current study.

This study aimed to optimize the extraction conditions for oil from *A. domesticus* (house crickets) to maximize yield. The effects of pressure, temperature, and extraction time on oil yield were investigated using Response Surface Methodology (RSM), and the suitable conditions for achieving the highest oil yield were determined. Additionally, the physicochemical properties (acid value (AV), percent free fatty acids (%FFAs), peroxide value (PV), iodine value (IV), and saponification value (SV)), total phenolic compound content, and antioxidant activities (ABTS and DPPH assay) of extracted cricket oil were analyzed.

2. Objectives

- 1) To determine the optimal temperature, pressure, and extraction time for maximizing cricket oil yield using SC-CO₂ extraction.
- 2) To study the influence of pressure, temperature, and time on the oil extraction process.
- 3) To analyze AV, %FFAs, PV, IV, SV, total phenolic compound content, and antioxidant activities of cricket oil extracted using SC-CO₂, as determined by ABTS and DPPH assays.

3. Materials and Methods

3.1 Sample preparation

Boiled house crickets were purchased from the Organic Agriculture Community Enterprise Group in Ban Mae Tat, Huai Sai, San Kamphang, Chiang Mai, Thailand. The house crickets were thawed and then dried in a tray dryer at 65°C for 17 h. Dried cricket contained 3.54% moisture, 16.01% crude fat, 11.63% crude fiber, 60.32% protein, 4.17% ash, and 4.33% carbohydrate. The dried crickets were then ground to reduce particle size, then packed in vacuum-sealed bags, and stored at room temperature before being extracted with SC-CO₂.

3.2 Supercritical CO₂ extraction

Oil extraction was carried out using a pilot-scale supercritical CO₂ extractor (Guangzhou Heavensent Industrial Co., Ltd., China). A sample of 500 g of ground cricket samples was placed into a 5 L extraction vessel. The extractions were conducted under conditions of temperatures of 40, 50, and 60°C, pressures of 175, 200, and 225 bar, and times of 1, 3, and 5 h. The oil extraction was done in triplicate for each condition. The extracted oil was collected and kept in amber bottles, including being flushed with nitrogen to remove oxygen before storage. Subsequently, the extracted oil was then stored in a refrigerator at 4°C for further analysis.



3.3 Experimental design

This research investigated the relationship between oil extraction efficiency from house crickets and the extraction conditions (pressure, temperature, and time). The experiment was designed using the Box-Behnken design (BBD). RSM was used to analyze the data in Minitab version 16 to determine the optimal extraction conditions. Fifteen treatments were obtained from three levels for each parameter: temperature (X_1) at 40, 50, and 60°C; pressure (X_2) at 175, 200, and 225 bar; and time (X_3) at 1, 3, and 5 h. The experimental data were fitted to a quadratic polynomial model in Equation (1).

$$Y = b_0 + \sum_{i=1}^k b_i X_i + \sum_{i=1}^k b_{ii} X_i^2 + \sum_{i \neq j=1}^k b_{ij} X_i X_j \quad (1)$$

where Y is predicted response, b_0 is constant term, b_i is linear regression coefficient for main variable effects, b_{ii} is the quadratic coefficient, and b_{ij} is the coefficient for interaction effects, while X_i and X_j are the uncoded independent variables. RSM was used to determine the optimal extraction conditions, which were validated by repeating the experiment three times and comparing the experimental values with the predicted results to evaluate the model's accuracy.

3.4 Determination of the chemical properties of extracted cricket oil

The chemical properties of the extracted cricket oil were examined using standard analytical methods to determine AV and %FFAs (Method 940.28), PV (Method 965.33), SV (Method 920.160), and IV (Method 920.158) (AOAC, 2000).

3.5 Determination of antioxidant activities

3.5.1 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay

To determine DPPH activity, following the method of Krzyckowska and Kozłowska (2017), 0.3 g of the cricket oil sample was mixed with 4.5 ml of methanol and vortexed for 15 s. The mixture was then centrifuged at 4500 rpm for 10 min. Next, 1000 μ l of the methanolic phase of the sample was pipetted and mixed with 100 μ l of a 1 mM DPPH solution (prepared in methanol). The resulting solution was then vortexed and incubated in the dark for 10 min. The solution was measured for the absorbance at 515 nm. The antioxidant activity of the oil sample was calculated by comparing it with the Trolox standard curve and is reported in mg eq Trolox/1 kg oil.

3.5.3 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay

The ABTS activity was measured following the method of Di Mattia et al. (2019), the ABTS solution was prepared by mixing 7 mM ABTS with 2.45 mM potassium persulfate solution and allowing it to react in the dark for 16 h at room temperature. The ABTS solution was then diluted with methanol to obtain an absorbance at 734 nm of 0.7 ± 0.02 . To determine ABTS activity, 0.3 g of the cricket oil sample was mixed with 4.5 ml of methanol and vortexed for 15 s, then centrifuged at 4500 rpm for 10 minutes. Next, 100 μ l of the methanolic phase was pipetted and mixed with 1000 μ l of the prepared ABTS solution. The solution was vortexed and incubated in the dark for 5 min. The absorbance at 734 nm was measured. The antioxidant activity of the oil sample was compared with the Trolox standard curve, and the results were reported as mg eq Trolox/1 kg of oil.

3.5.3 Total phenolic compound content

Total phenolics were determined following the method of Houshia and Qutit (2014) using the Folin-Ciocalteu reagent. The sample was prepared by mixing 1 g of cricket oil with 1 ml of hexane and 2 ml of 80% (v/v) methanol. The mixture was vortexed for 15 s and then centrifuged at 4500 rpm for 10 min. Next, 1 ml of the methanol phase was pipetted and mixed with 5 ml of 10% (v/v) Folin-Ciocalteu solution. This mixture was vortexed again and incubated in the dark for 5 minutes. Then, 1 ml of 20% (w/v) sodium carbonate (Na_2CO_3) solution was added. The mixture was vortexed and incubated in the dark at room temperature for 1 h. The absorbance of the solution was measured at 765 nm. The blank was prepared by adding 1 ml of distilled water to 5 ml of 10% (v/v) Folin-Ciocalteu solution and 1 ml of 20% (w/v) Na_2CO_3

[418]



solution. The amounts of phenolic compounds were compared with the gallic acid standard curve and reported as mg GAE/1 kg of oil.

3.6. Statistical analysis

Each extraction condition was performed in triplicate, and the quality analysis of the extracted oil was also conducted in triplicate. All data were analyzed using Analysis of Variance (ANOVA) and compared for differences in means using Duncan's Multiple Range Test (DMRT) at a confidence level of $p < 0.05$, using the SPSS statistical analysis program (Version 17.0). The estimated quadratic polynomial regression equation (Eq. (1)) was used to generate three-dimensional (3D) response surfaces and two-dimensional (2D) contour plots using Minitab version 16.

4. Results and Discussion

4.1 Fitting the oil yield model

The fifteen experimental treatments were carried out to determine the optimal conditions for enhancing high oil yield (as shown in Table 1). Extracted cricket oil yield ranged from 9.40 to 15.99%. The experimental data were analyzed using a quadratic polynomial model. ANOVA was performed to evaluate the significance of each coefficient in the model, and insignificant variables were excluded from the analysis (as shown in Table 2). The predictive model equation with uncoded variables for extracted cricket oil was determined using Minitab version 16, as follows:

$$Y = -70.4864 - 0.0816X_1 + 0.6898X_2 + 9.1547X_3 + 0.0033X_1^2 - 0.0012X_2^2 - 0.2638X_3^2 - 0.0011X_1X_2 - 0.0348X_2X_3 \quad (2)$$

where Y represents the extracted crude cricket oil (%), X_1 , X_2 , and X_3 correspond to the extraction temperature ($^{\circ}\text{C}$), pressure (bar), and time (h), respectively. Table 2 shows that the quadratic model is suitable for predicting the response during the optimization process, as indicated by a p -value of less than 0.05 and an adjusted R^2 value of 94.79%. This equation models the oil yield (Y) from *A. domesticus* using SC- CO_2 extraction, where pressure and time positively influence the yield ($p < 0.05$), whereas temperature has a slight negative effect ($p < 0.05$). The quadratic terms suggest that increasing each variable beyond a certain point may reduce yield, and the interaction terms indicate slight reductions when combining high pressure and long extraction times ($p < 0.05$). The p -value for lack of fit was 0.136 (>0.05), confirming that the quadratic polynomial model was effective in predicting the response variable in this study.

Table 1 Experimental design based on the Box-Behnken design and response variables

Treatment	Uncoded Independent Variables			Coded Independent Variables			Response variables
	Temperature (X_1 , $^{\circ}\text{C}$)	Pressure (X_2 , bar)	Time (X_3 , h)	Temperature	Pressure	Time	Crude Oil Yield (%)
1	40	175	3	-1	-1	0	13.58 \pm 0.00 ^{ef}
2	60	175	3	1	-1	0	14.24 \pm 0.01 ^{cd}
3	40	225	3	-1	1	0	15.83 \pm 0.54 ^a
4	60	225	3	1	1	0	15.34 \pm 0.41 ^{ab}
5	40	200	1	-1	0	-1	12.86 \pm 0.59 ^f
6	60	200	1	1	0	-1	13.87 \pm 0.67 ^{cd}
7	40	200	5	-1	0	1	15.14 \pm 0.00 ^{ab}
8	60	200	5	1	0	1	15.99 \pm 0.57 ^a
9	50	175	1	0	-1	-1	9.40 \pm 0.08 ^g
10	50	225	1	0	1	-1	14.66 \pm 0.00 ^{bc}
11	50	175	5	0	-1	1	15.54 \pm 0.19 ^a
12	50	225	5	0	1	1	13.83 \pm 0.00 ^{cd}
13	50	200	3	0	0	0	15.17 \pm 0.12 ^{ab}

[419]



Treatment	Uncoded Independent Variables			Coded Independent Variables			Response variables
	Temperature (X ₁ , °C)	Pressure (X ₂ , bar)	Time (X ₃ , h)	Temperature	Pressure	Time	Crude Oil Yield (%)
14	50	200	3	0	0	0	15.15±0.31 ^{ab}
15	50	200	3	0	0	0	15.23±0.50 ^{ab}

The crude oil yield (%) is presented as the mean \pm standard deviation, with different superscript letters indicating significant differences ($p < 0.05$)

Table 2: Analysis of variance for the polynomial model of crude oil yield

Source	DF	Seq SS	Adj SS	Adj MS	F	<i>p</i>
Model	8	74.7817	74.7817	9.3477	66.92	0.000
Linear	3	36.4965	36.4965	12.1655	87.09	0.000
Temperature	1	1.035	1.035	1.035	7.41	0.013
Pressure	1	11.8994	11.8994	11.8994	85.19	0.000
Time	1	23.5622	23.5622	23.5622	168.68	0.000
Square	3	13.3823	13.3823	4.4608	31.93	0.000
Temperature \times Temperature	1	1.6152	0.8267	0.8267	5.92	0.024
Pressure \times Pressure	1	3.5436	4.3993	4.3993	31.49	0.000
Time \times Time	1	8.2235	8.2235	8.2235	58.87	0.000
Interaction	2	24.9028	24.9028	12.4514	89.14	0.000
Temperature \times Pressure	1	0.6472	0.6472	0.6472	4.63	0.043
Pressure \times Time	1	24.2556	24.2556	24.2556	173.64	0.000
Residual Error	21	2.9334	2.9334	0.1397		
Lack-of-Fit	4	0.9469	0.9469	0.2367	2.03	0.136
Pure Error	17	1.9865	1.9865	0.1169		
Total	29	77.7151				

$R^2 = 96.23\%$, $R^2_{\text{pred.}} = 92.47\%$, $R^2_{\text{adj.}} = 94.79\%$

4.2 Analysis of the response surface of extracted oil yield

Response surface plots were generated to analyze the effects of extraction temperature, pressure, and time on crude oil yield by illustrating the interactions between two variables while holding one variable constant. The response surface plot illustrates the interaction between pressure and temperature on crude oil yield (shown in Figure 1a). The plot shows that an increase in pressure significantly enhances crude oil yield. Moreover, an increase in temperature, in combination with higher pressure, led to a further increase in oil yield. This could be due to high pressure leading to a denser CO₂ phase, which increases CO₂ density and reduces intermolecular distances, thereby improving the solvent's dissolving ability and facilitating oil extraction from the solid matrix (Purschke et al., 2017). Additionally, a higher extraction temperature increases vapor pressure and enhances solvent diffusion, contributing to improved extraction efficiency (Wang et al., 2019). These combined factors were found to enhance oil extraction. However, at pressures above 200 bar, the surface plot showed a different trend, with oil yield increasing slightly at lower temperatures. This suggests that while pressure is the dominant factor in oil recovery, excessive heat may have reduced the effectiveness of the oil extraction process. The statistical model (Eq. (2)) further supports this observation, indicating a negative coefficient for temperature, implying that higher temperatures could reduce extraction efficiency under certain conditions ($p < 0.05$). Overall, the surface plot confirms that pressure plays a crucial role in enhancing oil yield, while extraction temperature may have a negative impact extraction efficiency under specific conditions.

Figure 1b illustrates the relationship between extraction temperature and time on oil yield. The plot shows that both higher temperatures and longer extraction times enhance oil yield. However, extraction time has a stronger effect, as indicated in Eq. (2) (p -value < 0.05).

Figure 1c demonstrates the relationship between extraction pressure and extraction time. A similar trend was observed in the oil extraction from *T. molitor* L. larvae and black soldier fly larvae, where the yield

[420]



increased with increasing pressure (Kim et al., 2019; Purschke et al., 2017). However, prolonged extraction time at high pressure does not significantly affect oil yield. This suggests that the extraction process may reach a saturation point, where most extractable oil has already been dissolved under these conditions. Extending the extraction time beyond this point offers no additional benefit.

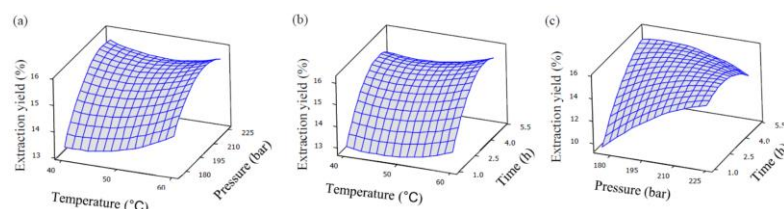


Figure 1 Response surface plots of oil yield: (a) The effect of temperature and pressure on oil yield, showing an increase in crude oil yield with higher pressure and temperature. (b) The effect of temperature and time on oil yield, indicating that higher temperatures and longer extraction times enhance oil yield. (c) The effect of pressure and time on oil yield, demonstrating that higher pressure improves oil yield, prolonged extraction time at high pressure does not further enhance the yield

4.3 Optimization and verification of cricket oil extraction process model

The predicted optimal condition was 60°C, 181.25 bar, and 5 h, with a predicted crude oil yield of 16.37% (as shown in Figure 2). To validate the reliability of the experimental model, extraction was performed at 60°C, 175 bar, and 5 h, as 175 bar is close to the predicted pressure. The experimental yield was 16.20%, compared to the predicted yield of 16.37%. This result confirms that the model is suitable for predicting oil yield under these research conditions.

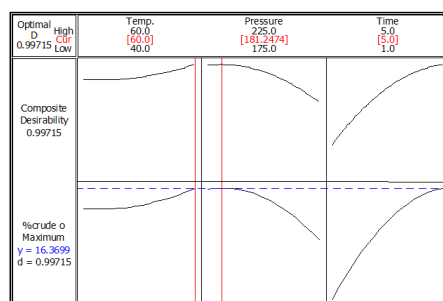


Figure 2 Two-dimensional plot showing the optimal conditions for cricket oil extraction at 60°C, 181.25 bar, and 5 h, with a predicted crude oil yield of 16.37%

4.4 Response surface analysis of physicochemical properties of extracted cricket oil

Figure 3 demonstrates the influence of extraction temperature, pressure, and extraction time on physicochemical properties, including AV, %FFAs, PV, IV, and SV.

Figure 3a shows that both pressure and temperature significantly affect AV. The AV of the extracted cricket oil ranged from 2.41 to 5.71 mg KOH/g oil. As shown in Figure 3a, at a constant temperature, a decrease in pressure results in an increase in the acid value, indicating that SC-CO₂ can extract oil with lower levels of free fatty acids. A similar trend to that reported by Dunford and Temelli (1997) in canola oil extraction was observed. As pressure decreased, the solubility of triglycerides in SC-CO₂ was reduced due to the decrease in CO₂ density, resulting in a selective shift of SC-CO₂ toward lower molecular weight free fatty acids. The %FFAs results shown in Figure 3b were similar to those of the AV.

The experimental results showed that the PV ranged from 18.76 to 70.92 (meqO₂/kg oil), with both pressure and temperature playing crucial roles in influencing PV (as shown in Figure 3c). It was evident that the PV increased between 40-50°C as pressure rose from 175 to 200 bar. Increasing extraction temperature



accelerated oxidation reactions, thereby promoting oxidation and increasing the peroxide value, as described by Subroto et al. (2017). However, a change in trend was observed, where the PV slightly decreased at higher pressures between 50–60°C.

IV is a measure of the degree of unsaturation in the fatty acids present in oil, with a higher IV indicating a greater level of unsaturation (Pulassery et al., 2022). Trends in IV under varying pressure and temperature conditions show only minor changes (as shown in Figure 3d). The IV increased as pressure and extraction time increased. At pressures below 200 bar, the IV slightly decreased with increasing temperature, likely due to the reduced solubility of unsaturated fatty acids (Wiriyaacharee et al., 2024). The extracted cricket oil exhibited an IV range of 67.04–77.50 g I₂/100 g of oil, which is comparable to soya bean oil (high oleic acid) (75–95 g I₂/100 g oil), as reported by the Codex Alimentarius Commission (2024).

The saponification value (SV) reflects the molecular weight or chain length of fatty acids in oil (Pulassery et al., 2022). The SV followed the same trend as IV, increasing with pressure. Additionally, SV increases as the extraction temperature rises, while keeping the pressure constant (as shown in Figure 3e). The SV of cricket oil ranged from 172.18 to 202.98 mg KOH/g oil, which was comparable to that of common edible oils, including soya bean oil (high oleic acid) (188–192 mg KOH/g oil), rice bran oil (180–199 mg KOH/g oil), rapeseed oil (168–181 mg KOH/g oil), and camellia seed oil (187–199 mg KOH/g oil) (Codex Alimentarius Commission, 2024). House cricket oil demonstrated good quality, as indicated by its IV and SV values. The SV values were within the typical range of edible oils, suggesting similar functional chain lengths and suitability for food applications.

4.5 Response surface analysis of Total Phenolic Content, DPPH, and ABTS radical scavenging activities of extracted cricket oil

Figure 4 demonstrated the influence of extraction temperature, pressure, and extraction time on the total phenolic content (TPC), DPPH, and ABTS radical scavenging activities of cricket oil. The TPC, DPPH, and ABTS radical scavenging activities in cricket oil ranged from 19.14 to 51.06 mg GAE/1 kg oil, 3.21 to 50.37 mg eq Trolox/1 kg oil, and 35.42 to 151.40 mg eq Trolox/1 kg oil, respectively.

According to Figure 4a, at a constant extraction time and temperatures between 40–50°C, TPC decreased as both temperature and pressure increased. This finding suggests that the solubility of phenolic compounds may be lower at higher pressures under moderate temperatures (40–50°C). Meanwhile, TPC increased at high pressures between 50 and 60°C, suggesting that the increased vapor pressure of phenolic compounds and the higher SC-CO₂ density at high pressure enhance their solubility (Sato et al., 2019). The observed trends indicate that at lower temperatures (40–50°C), higher pressure did not enhance TPC extraction, while at higher temperatures (50–60°C), pressure positively influences TPC solubility. However, as extraction time increased, TPC was observed to decrease.

The scavenging activity of DPPH increased with temperature at pressures below 210 bar, due to improved extraction efficiency. However, at pressures above 210 bar, DPPH decreased slightly, suggesting that higher pressure may negatively affect DPPH. This could be due to the loss of some beneficial compounds and the extraction of different compounds (Ahmed et al., 2022). Additionally, for longer extraction time, the scavenging activity of DPPH decreased further. This contrasts with TPC, where higher pressure at higher temperatures enhanced solubility. The difference could be due to the distinct properties of DPPH and TPC, with higher pressure promoting TPC solubility but possibly reducing the scavenging activity of DPPH over time.

The ABTS scavenging activity decreased as the temperature increased from 40°C to 50°C. However, between 50°C and 60°C, the scavenging activity increased with higher pressure. At high pressure, ABTS scavenging activity also increased. However, with longer extraction time, ABTS slightly decreased. These findings suggest that higher pressure may enhance the solubility of antioxidants in SC-CO₂, improving extraction efficiency. This trend suggested that pressure may play a significant role in increasing ABTS scavenging activity, similar to the results observed for TPC. The difference between DPPH and ABTS indicated that the ability to scavenge radicals may be due to different antioxidant compounds being extracted under different conditions.

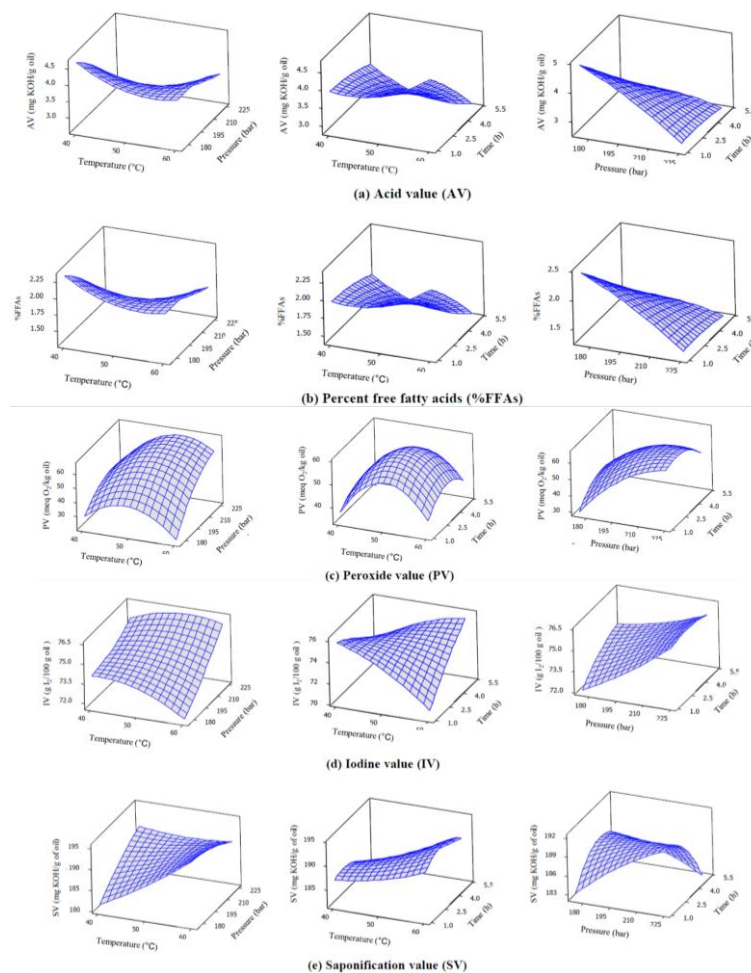


Figure 3 Response surface plots showing the effects of extraction temperature, pressure, and time on the following properties of cricket oil: (a) acid value (AV), (b) free fatty acids (FFAs), which decreased at higher pressures, (c) peroxide value (PV), which increased with higher temperatures and pressures, (d) iodine value (IV), which decreased with higher temperatures but increased with higher pressure, and (e) saponification value (SV), which increased with higher temperatures

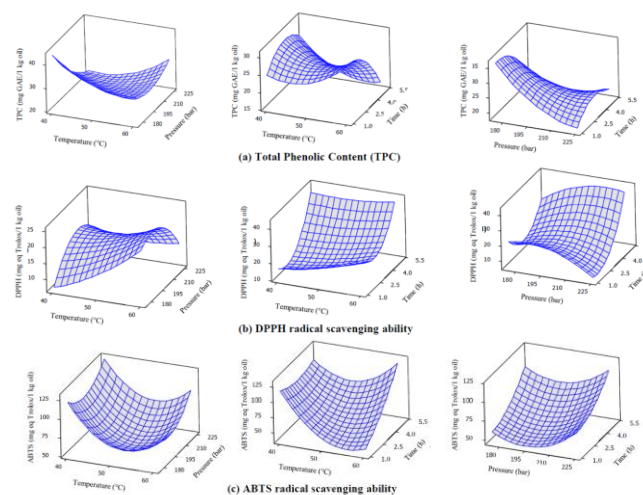


Figure 4 Response surface plots showing the effect of extraction temperature, pressure, and time on (a) Total Phenolic Content (TPC), which increased with higher temperature and pressure (b) DPPH radical scavenging activity, which decreased at higher pressure (c) ABTS radical scavenging activity, which increased with higher temperature and pressure

4.6. Limitations and future research

While the findings are promising, their practical application for large-scale production remains to be explored. Optimizing SC-CO₂ extraction conditions to increase yield and reduce costs could make it more commercially viable for large-scale oil extraction. The scalability of this method, along with its potential to replace traditional solvent-based methods, offers a promising avenue for more sustainable and efficient oil production in the food and nutraceutical industries. Although this study provides valuable insights into the SC-CO₂ extraction of cricket oil, there are several limitations to consider. The small-scale laboratory conditions may not fully represent industrial-scale processes, and variations in environmental factors, such as temperature and pressure fluctuations, could affect the consistency of the results. Future studies should focus on scaling up the extraction process and evaluating its feasibility under real-world conditions to better assess its commercial potential.

5. Conclusion

SC-CO₂ was employed to extract oil from house crickets. The extraction parameters indicated that both pressure and time positively influence yield ($p < 0.05$), while higher values may reduce efficiency. Temperature has a minor negative effect, but may have less impact that diminishes at higher levels ($p < 0.05$). Interaction terms suggest that combining high temperature, pressure, or prolonged time may not enhance yield. This model helps optimize extraction conditions ($p < 0.05$). The optimal experimental conditions were 60°C, 175 bar, and 5 h, which resulted in a high oil yield of 16.20%. The AV of the extracted cricket oil ranged from 2.41 to 5.71 mg KOH/g oil. Additionally, the PV ranged from 18.76 to 70.92 meq O₂/kg oil and slightly decreased at higher pressure. The IV was relatively high, ranging from 67.04 to 77.50 g I₂/100 g oil, and SV ranged from 172.18 to 202.98 mg KOH/g oil. Moreover, cricket oil demonstrated antioxidant activity, as evidenced by its DPPH and ABTS radical scavenging activities, as well as its total phenolic compound content. These results suggest that they provide valuable data for further research in developing house cricket oil for applications in the food and nutraceutical industries with the SC-CO₂ extraction method.

6. Acknowledgements

The authors gratefully acknowledge the Faculty of Agro-Industry, Chiang Mai University, Chiang Mai, for supporting in providing laboratory equipment, research facilities, and additional assistance.

[424]



7. References

- Ahmed, H. Y., Kareem, S. M., Atef, A., Safwat, N. A., Shehata, R. M., Yosri, M., ... & Elsaid, F. G. (2022). Optimization of supercritical carbon dioxide extraction of *Saussurea costus* oil and its antimicrobial, antioxidant, and anticancer activities. *Antioxidants*, 11(10), Article 1960. <https://doi.org/10.3390/antiox11101960>
- AOAC. (2000). *Official Methods of Analysis* (seventeenth). Association of Official Analytical Chemists.
- Brogan, E. N., Park, Y.-L., Shen, C., Matak, K. E., & Jaczynski, J. (2023). Characterization of lipids in insect powders. *LWT*, 184, 115040. <https://doi.org/10.1016/j.lwt.2023.115040>
- Megido, R. C., Gierts, C., Blecker, C., Brostaux, Y., Haubruge, É., Alabi, T., & Francis, F. (2016). Consumer acceptance of insect-based alternative meat products in Western countries. *Food quality and preference*, 52, 237-243. <https://doi.org/10.1016/j.foodqual.2016.05.004>
- Codex Alimentarius Commission. (2024). *Standard for Named Vegetable Oils* (CXS 210-1999). Food and Agriculture Organization of the United Nations/World Health Organization. https://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCXS%2B210-1999%252FCXS_210e.pdf
- Chaiyana, W., Inthorn, J., Somwongin, S., Anantaworasakul, P., Sopharadee, S., Yanpanya, P., ... & Kanjanakawinkul, W. (2024). The fatty acid compositions, irritation properties, and potential applications of teleogryllus mitratus oil in nanoemulsion development. *Nanomaterials*, 14(2), Article 184. <https://doi.org/10.3390/nano14020184>
- Di Mattia, C., Battista, N., Sacchetti, G., & Serafini, M. (2019). Antioxidant activities in vitro of water and liposoluble extracts obtained by different species of edible insects and invertebrates. *Frontiers in nutrition*, 6, Article 438996. <https://doi.org/10.3389/fnut.2019.00106>
- Dunford, N. T., & Temelli, F. (1997). Extraction conditions and moisture content of canola flakes as related to lipid composition of supercritical CO₂ extracts. *Journal of Food Science*, 62(1), 155-159. <https://doi.org/10.1111/j.1365-2621.1997.tb04389.x>
- Fornari, T., Vázquez, L., Villanueva-Bermejo, D., Hurtado-Ribeira, R., Martín Hernández, D., & Martin, D. (2023). Effect of moisture and oil content in the supercritical CO₂ defatting of *Hermetia illucens* larvae. *Foods*, 12(3), Article 490. <https://doi.org/10.3390/foods12030490>
- Houshia, O. J., Qutit, A., Zaid, O., Shqair, H., & Zaid, M. (2014). Determination of total polyphenolic antioxidants contents in West-Bank olive oil. *Journal of Natural Sciences Research*, 4(15), 71-79.
- Kim, S. W., Jung, T. S., Ha, Y. J., Gal, S. W., Noh, C. W., Kim, I. S., ... & Yoo, J. H. (2019). Removal of fat from crushed black soldier fly larvae by carbon dioxide supercritical extraction. *Journal of Animal and Feed Sciences*, 28(1), 83-88. <https://doi.org/10.22358/jafs/105132/2019>
- Krzyczkowska, J., & Kozłowska, M. (2017). Effect of oils extracted from plant seeds on the growth and lipolytic activity of *Yarrowia lipolytica* yeast. *Journal of the American Oil Chemists' Society*, 94(5), 661-671. <https://doi.org/10.1007/s11746-017-2975-1>
- Laroche, M., Perreault, V., Marciniak, A., Gravel, A., Chamberland, J., & Doyen, A. (2019). Comparison of conventional and sustainable lipid extraction methods for the production of oil and protein isolate from edible insect meal. *Foods*, 8(11), Article 572. <https://doi.org/10.3390/foods8110572>
- Magara, H. J., Niassy, S., Ayieko, M. A., Mukundamago, M., Egonyu, J. P., Tanga, C. M., ... & Ekesi, S. (2021). Edible crickets (Orthoptera) around the world: distribution, nutritional value, and other benefits—a review. *Frontiers in nutrition*, 7, Article 537915. <https://doi.org/10.3389/fnut.2020.537915>
- Mercola, J., & D'Adamo, C. R. (2023). Linoleic acid: A narrative review of the effects of increased intake in the Standard American Diet and Associations with Chronic Disease. *Nutrients*, 15(14), Article 3129. <https://doi.org/10.3390/nu15143129>
- Pilco-Romero, G., Chisaguano-Tonato, A. M., Herrera-Fontana, M. E., Chimbo-Gándara, L. F., Sharifi-Rad, M., Giampieri, F., ... & Álvarez-Suárez, J. M. (2023). House cricket (*Acheta domesticus*): A



- review based on its nutritional composition, quality, and potential uses in the food industry. *Trends in Food Science & Technology*, 142, 104226. <https://doi.org/10.1016/j.tifs.2023.104226>
- Pulassery, S., Abraham, B., Ajikumar, N., Munnalath, A., & Yoosaf, K. (2022). Rapid iodine value estimation using a Handheld Raman Spectrometer for on-site, reagent-free authentication of edible oils. *ACS Omega*, 7(11), 9164–9171. <https://doi.org/10.1021/acsomega.1c05123>
- Purschke, B., Stegmann, T., Schreiner, M., & Jäger, H. (2017). Pilot-scale supercritical CO₂ extraction of edible insect oil from *Tenebrio molitor* L. larvae – Influence of extraction conditions on kinetics, defatting performance and compositional properties. *European Journal of Lipid Science and Technology*, 119(2), Article 1600134. <https://doi.org/10.1002/ejlt.201600134>
- Sato, T., Ikeya, Y., Adachi, S. I., Yagasaki, K., Nihei, K. I., & Itoh, N. (2019). Extraction of strawberry leaves with supercritical carbon dioxide and entrainers: Antioxidant capacity, total phenolic content, and inhibitory effect on uric acid production of the extract. *Food and Bioprocess Processing*, 117, 160–169. <https://doi.org/10.1016/j.fbp.2019.07.003>
- Schoss, K., & Glavač, N. K. (2024). Supercritical CO₂ extraction vs. hexane extraction and cold pressing: Comparative analysis of seed oils from six plant species. *Plants*, 13(23), 3409. <https://doi.org/10.3390/plants13233409>
- Subroto, E., Widjojokusumo, E., Veriansyah, B., & Tjandrawinata, R. R. (2017). Supercritical CO₂ extraction of candlenut oil: Process optimization using Taguchi orthogonal array and physicochemical properties of the oil. *Journal of Food Science and Technology*, 54(5), 1286–1292. <https://doi.org/10.1007/s13197-017-2542-7>
- Wang, W., Han, S., Jiao, Z., Cheng, J., & Song, J. (2019). Antioxidant activity and total polyphenols content of camellia oil extracted by optimized supercritical carbon dioxide. *Journal of the American Oil Chemists' Society*, 96(11), 1275–1289. <https://doi.org/10.1002/aocs.12285>
- Wiriacharee, P., Chalermchat, Y., Siriwoharn, T., Jirattananarangsri, W., Tangjaidee, P., Chaipoot, S., Phongphisutthinant, R., Pandith, H., & Muangrat, R. (2024). Utilizing supercritical CO₂ for bee brood oil extraction and analysis of its chemical properties. *Foods*, 13(16), Article 2486. <https://doi.org/10.3390/foods13162486>