A novel process for the aqueous extraction of oil from *Camellia oleifera* seeds and its antioxidant activity

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**SUMMARY**

A novel process for the aqueous extraction of oil from *Camellia oleifera* seed and its antioxidant activity

Aqueous extraction is a promising green alternative to hexane extraction. This study used a salt effect-aided aqueous extraction process (AEP-SE) for extracting *Camellia oleifera* seed oil (COSO) to improve oil extractability and avoid emulsification in the aqueous system. The highest oil extractability rate of 88.8% was obtained under 1.48 mol L⁻¹ sodium carbonate, a solution-to-flour ratio of 3.85, and 3.23h of extraction time with the quality of the aqueous system-extracted oil being similar to those of a commercial sample of COSO and hexane-extracted oil in terms of color, iodine value and saponification value, although its moisture content was higher. Furthermore, the free fatty acid content of the aqueous system-extracted oil was lower than that of the solvent-extracted oil. The values of the inhibitory concentration at 50% of oil obtained by AEP-SE and organic solvent extraction as measured by DPPH scavenging activity essay, were 2.27 mg/mL and 3.31 mg/mL. AEP-SE is therefore a promising environmentally friendly method for the large-scale preparation of COSO.

**KEY-WORDS:** Antioxidation activity – Aqueous process – *Camellia oleifera* – Seed oil.

1. **INTRODUCTION**

The shrub *Camellia oleifera* originates from China. It is distributed in 18 provinces, cities, and municipalities in the southern region thereof, including Hunan, Jiangxi and Fujian, and is less abundant in the northern region of Southeast Asia. *C. oleifera* seed oil (COSO) is a high-quality edible oil that has been suggested to protect the liver against CCl₄-induced oxidative damage (Tasan et al., 2011; Lee et al., 2007; Ma et al., 2011). COSO is one of the four edible tree oils (with the three others being palm oil, olive oil, and coconut oil) (Zhang et al., 2007a; Zhang et al., 2007b). Long and Wang labeled COSO as “eastern olive oil” (Long and Wang, 2008), because it contains abundant unsaturated fatty acids, consisting of oleic acid and linoleic acid. Olive oil contains approximately 77% monounsaturated fatty acids and several compounds that can reduce the risk of cancer (Chen et al., 2009; Ye et al., 2001). Due to its high oleic acid content and high levels of natural antioxidation (phenols and tocopherol), COSO is highly resistant to peroxidation, thus forming few free radicals (Ye et al., 2001).

COSO has been traditionally recovered by pressing followed by solvent extraction, mainly with n-hexane. Hexane extraction has substantial safety issues, including the risk of fire, and entail high costs for plants equipped to handle the solvents. Moreover, the emission of volatile n-hexane into the atmosphere contributes to the formation of ozone. In light of these considerations, the vegetable oil industry is actively looking for an alternative process (Fang et al., 2009; Moura et al., 2008; Latif and Anwar, 2011).

Aqueous extraction processing (AEP) is a promising green alternative to hexane extraction.
Although recent advances in AEP techniques have increased the recovery of free oil to 85% (Moura et al., 2008; Zhang et al., 2012; Sant'Anna et al., 2003), AEP yields from industrial hexane extraction processes are still less than typical. AEP uses water as an extraction medium, which dissolves soluble cellular materials and allows for the release of oil into the bulk liquid phase, from which the oil can be recovered by centrifugation, resulting in a cream emulsion which can be broken down to recover free oil (Moura et al., 2008; Chabrand et al., 2008; Chabrand and Glatz, 2009). Approximately 10-15% of the oil released from the solid fraction also remains in the aqueous fraction as an emulsion which is stable toward creaming (Moura et al., 2008). Rosenthal et al reported that oil yield is directly proportional to the inverse of flour particle size, which they attributed to cellular disruption oil release. The immiscible nature of AEP systems suggests a potential role for de-emulsification to release more free oil (Rosenthal and Niranjan, 1998). The immiscible nature and known mechanisms of oil release of the oil/water system are intrinsically different from those of hexane extraction processes. Campbell et al., (2009) showed that emulsification is an important extraction mechanism that reduces the size of the droplets and allows the cream emulsion to increase its free oil yield (Hammourjai et al., 2000). The solid-liquid ratio, extraction time, temperature, pH and agitation rate are important parameters for extraction (Campbell and Glatz, 2009; Nikiforidis and Kiosseoglou, 2009).

In this study, we evaluated an aqueous process for the extraction of *C. oleifera* oil from COS kernels using a sodium carbonate solution instead of water as the extraction medium. This important step prevented emulsion formation in the aqueous system, which resulted in a very high oil yield. To the best of our knowledge, this novel process for COSO extraction has not been previously reported.

### 2. MATERIALS AND METHODS

#### 2.1. Materials and reagents

COSs were purchased from a market in the Youxi Country Fujian Province, China. Each COS was successively passed through a 16-mesh sieve (aperture size, 1000mm) to remove foreign materials. After sieving, the COS was stored in plastic bags at −18°C, and thawed in a refrigerator at 4°C a day before use. The composition of the COS is given in Table 1, and the methods used to determine it are described in the following sections.

A sodium carbonate solution was prepared by dissolving 40-250 g of sodium carbonate in 1 L deionized water. All other chemicals used were of analytical grade.

#### 2.2. Aqueous extraction process

The process was operated by batch using a 2-L jacketed glass reactor vessel held at setting temperature by a circular water bath and agitated by a stirrer. The key stages of the procedure are shown in Fig. 1.

For most experiments, the extraction was carried out by dispersing 10 g of COS flour into 1 L of the solution, giving a solution-to-flour ratio of 4:1(v/w). The mass of the flour obtained in other experiments was dependent on the chosen value of the solution-to-flour ratio. The mixture was stirred, and the water bath temperature was maintained from 50°C to 90°C.

#### 2.3. Solid-liquid separation

After extraction, the mixture was centrifuged at 16800 × g for 30 min to separate the crude oil, supernatant, and solid phase (or defatted flour) at ambient temperature. The crude oil from the top layer was collected with a pipette and transferred to a beaker tube. The defatted flour, which consisted of an upper layer of sedimented components, a lower layer of sedimented components, and a lower layer of undestroyed cell debris was mixed, weighed, and sampled for the determination of moisture content and total dry defatted flour. The remaining defatted flour was oven-dried overnight at 105 degrees and analyzed for residual oil.

#### 2.4. Hexane extraction

A Soxhlet extractor was heated in a water bath. Each thimble was filled with 5 g of the COS flour...
and extract for 8 h. The micelle was evaporated in a rotary evaporator. The oil obtained was dried in a hot air oven at 105 degrees for 30 min to eliminate residual hexane and collected in a sealed glass bottle.

2.5. Extractability

Extractability was calculated as the difference between the oil content of the COS flour before aqueous extraction and the residual oil in the defatted flour. It was expressed as a percentage of the initial oil content. Each extraction experiment was repeated three times, and the error bars shown in the figures indicate the range of values obtained.

2.6. Analytical methods

Moisture and volatile content were determined as weight loss at 103 ± 2 degrees for 3 h accorded by IUPAC, and the crude oil in the COS kernel and residual oil in the defatted flour were extracted using the Soxhlet method. Nitrogen content was analyzed by the Kjeldahl method and a factor of 5.595 was used to estimate the protein content. The flour was burnt in a furnace at 600 degrees for 2 h to determine the ash content. The oil obtained by aqueous extraction was analyzed for its (i) color with a Lovibond colorimeter, as well as (ii) free fatty acid content, iodine value, peroxide value and saponification value and fatty acid composition by methods recommended by the AOCS.

2.7. Response surface method analysis

The range of values for each factor was determined based on the single-factor experimental results. The response surface method (RSM) was used to analyze the effects of each factor on extractability. Three factors were selected as independent variables, whereas extractability was defined as the dependent variable.

2.8. DPPH scavenging activity

The anti-oxidation of aqueous system-extracted COSO was studied using the DPPH scavenging activity assay. The oil obtained by organic solvent extraction (OSE) was used for comparison. Ascorbic acid (Sigma-Aldrich) was used for reference purposes. Standard ascorbic acid was prepared with ethanol. The sample solution (0.1 mL) was combined with 1.4 mL of ethanol, and a 1 mL of 0.04 mg mL⁻¹ DPPH solution was subsequently added. The mixture was shaken vigorously and placed in a Shimadzu UV-2100 spectrophotometer (Shimadzu, Japan) to monitor the decrease in absorbance at 517 nm. Monitoring was continued for 70 min until the reaction reached a plateau. The inhibition percentage of DPPH was calculated according to the following formula:

\[ \text{Inhibition} (\%) = \left( \frac{I_{\text{control}} - I_{\text{sample}}}{I_{\text{control}} - I_b} \right) \times 100\% \]

where \( I_{\text{control}} \), \( I_{\text{sample}} \) and \( I_b \) represent the absorbance levels of the oil solution controls and background, respectively. The inhibition rate was plotted against the sample concentration. A logarithmic regression curve was established to calculate the inhibitory concentration at 50% (IC50), which was the amount of sample necessary to lower the DPPH activity by 50% compared with the untreated control.

3. RESULTS AND DISCUSSION

3.1. Effect of sodium carbonate solution concentration on oil extractability

The effect of sodium carbonate concentration on oil extractability was investigated and the results are reported in Fig. 2. Oil extractability increased substantially with the concentration of Na₂CO₃ solutions (Fig. 2) suggesting that COSO can be extracted more effectively under salt effect (SE) conditions by Na₂CO₃. The highest extraction yield was achieved at 1.4 M, at which approximately 82% of the oil was extracted avoiding emulsification in the aqueous system.

3.2. Effect of extraction time on oil extractability

The effect of extraction time on oil extractability is illustrated in Fig. 3. Oil extractability increased marginally when extraction time increased. An extraction time of 3 h was therefore selected for the process.

3.3. Effect of solution-to-flour ratio on oil extractability

The results of varying the solution-to-flour ratio in different proportions are shown in Fig. 4.

![Effect of Na₂CO₃ solution concentrations on oil extractability.](image)
3.6. Optimization of aqueous extraction by RSM

The aqueous extraction was optimized by RSM according to the Box-Behnken design. The effects of three independent parameters, namely, sodium carbonate concentration, solution-to-flour ratio, and extraction time were considered. The oil extractability results are listed in Table 2.

The data were analyzed using response surface models. Analysis of variance (ANOVA) revealed that the models significantly represented the experimental data, for which the coefficient of multiple determinations ($R^2$) of the responses of the extraction of oil was 0.94. ANOVA was also used to evaluate the significance of the models and their parameters (Table 3). A small $P$ value indicated a significant effect on oil extractability.

3.4. Effect of extraction temperature on oil extractability

The effect of extraction temperature on oil extractability is illustrated in Fig. 5.

No substantial effect of temperature was observed when the extraction temperature was raised from 40 degrees to 90 degrees at the same concentration of sodium carbonate (1.4M). However, at 90 degrees, oil extractability decreased due to water evaporation. Therefore, a temperature of 70 degrees was deemed satisfactory for oil extraction.

3.5. Effect of agitation speed on oil extractability

The effect of agitation speed on oil extractability is shown in Fig. 6. Speed variation had no substantial effect on oil extraction. When the experiment was carried out at the lowest speed of 100 rpm, solids were observed to settle slowly; the speed was therefore increased occasionally to ensure homogeneity. The results suggested that high agitation speeds are not needed.

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Oil extractability was significantly affected by the solution-to-flour ratio (Fig. 4). At the higher ratios, the higher viscosity of the mixture made it difficult to maintain mixture homogeneity. From these experiments, it is clear that a ratio in the range of 4-5.5 is adequate for the process.

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response variables. Significant interaction terms indicated that the interaction between the factors significantly affected oil extractability. In the residual part, the lack of fit was not significant, indicating that the model fit well. Thus, the sodium carbonate concentration showed significant effects on the extraction of oil ($P < 0.01$). The quadratic terms demonstrated that the solution-to-flour ratio significantly affected oil extractability ($P < 0.05$) as did concentration and extraction time ($P < 0.01$). Furthermore, the interactions between the sodium carbonate concentration and solution-to-flour ratio had significant effects on oil extractability.

As for the optimization of COSO yield, the responses were analyzed using Design Expert 7.0 software. A quadratic polynomial regression model was assumed for predicting responses. The model proposed for each response of $Y$ was as follows:

$$
Y = 86.92 + 6.73X_1 - 0.91X_2 + 3.32X_3
-3.73X_1X_2 + 2.56X_1X_3 - 9.36X_2^2
-5.19X_3^2 - 7.73X_1^2
$$

(1)

The goodness of fit of the model was evaluated by the coefficient of determination $R^2 (0.94)$.

Many parameters can influence oil extractability. Eq. (1) shows that COSO extractability has a complex relationship with independent variables that encompass both first and second-order polynomials and may have more than one maximum point. The best way of expressing the effect of any parameter on the yield within the experimental space under investigation was to generate response surface plots of the equation. Contour plots and response surface curves showing predicted response surface of oil extractability as a function of the solution-to-flour ratio and Na$_2$CO$_3$ solution concentration are depicted in Fig. 7. The three-dimensional response surfaces are plotted as a function of the interactions of any two of the variables by holding the other one at middle value. Both plots in Fig. 7 show the relationships with respect to the effects of each significant variable.

Table 3 and Fig. 7 show variables $X_1$ and $X_2$ with large regression coefficients. Panels (a) and (b) of Fig. 7 specifically show that increasing sodium carbonate concentration led to an increase in oil extractability at a low ratio but resulted in a slight decrease at a higher ratio. The same effects were observed for the solution-to-flour ratio. Since the oil extractability, in relation to the solution-to-flour ratio and that in relation to the sodium carbonate solution, can represent the efficiency of the aqueous process, the optimization of the process...
The moisture content of the oil obtained by the aqueous process was considerably higher than those of other samples. Hexane-extracted oil also exhibited a substantially higher content of free fatty acid. These findings indicate that the COSO used in all experiments contained some free fatty acid due to high temperature during organic extraction and processing (Tasan et al., 2011). The lower free fatty acid content of the oil obtained by the aqueous process is probably due to some neutralization of the free fatty acid by Na$_2$CO$_3$ during extraction.

3.8. Fatty acid composition of COSO

The fatty acid composition of COSO obtained by AEP and OSE were analyzed by GC, and the results are shown in Table 5.

<table>
<thead>
<tr>
<th>Source</th>
<th>Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F Value</th>
<th>P-value (Prob &gt; F)</th>
<th>Significant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
<td>1180.9696</td>
<td>9</td>
<td>131.2188</td>
<td>10.9902</td>
<td>0.0023 **</td>
<td></td>
</tr>
<tr>
<td>$X_1$</td>
<td>304.31576</td>
<td>1</td>
<td>304.3157</td>
<td>25.4880</td>
<td>0.0015 **</td>
<td></td>
</tr>
<tr>
<td>$X_2$</td>
<td>6.67956</td>
<td>1</td>
<td>6.6795</td>
<td>0.5594</td>
<td>0.4789</td>
<td></td>
</tr>
<tr>
<td>$X_3$</td>
<td>61.96136</td>
<td>1</td>
<td>61.9613</td>
<td>5.1895</td>
<td>0.0568</td>
<td></td>
</tr>
<tr>
<td>$X_1X_2$</td>
<td>55.57706</td>
<td>1</td>
<td>55.5770</td>
<td>4.6548</td>
<td>0.0479 *</td>
<td></td>
</tr>
<tr>
<td>$X_1X_3$</td>
<td>14.22556</td>
<td>1</td>
<td>14.2255</td>
<td>1.1914</td>
<td>0.3112</td>
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</tr>
<tr>
<td>$X_2X_3$</td>
<td>0.1225</td>
<td>1</td>
<td>0.1225</td>
<td>0.0102</td>
<td>0.9222</td>
<td></td>
</tr>
<tr>
<td>$X_1^2$</td>
<td>340.34546</td>
<td>1</td>
<td>340.3454</td>
<td>28.5057</td>
<td>0.0011 **</td>
<td></td>
</tr>
<tr>
<td>$X_2^2$</td>
<td>101.6042</td>
<td>1</td>
<td>101.6042</td>
<td>8.5099</td>
<td>0.0224 *</td>
<td></td>
</tr>
<tr>
<td>$X_3^2$</td>
<td>198.9888</td>
<td>1</td>
<td>198.9887</td>
<td>16.6663</td>
<td>0.0047 **</td>
<td></td>
</tr>
<tr>
<td>Residual</td>
<td>83.57678</td>
<td>7</td>
<td>11.9395</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lack of Fit</td>
<td>78.6399</td>
<td>3</td>
<td>26.2132</td>
<td>21.2387</td>
<td>0.064</td>
<td></td>
</tr>
<tr>
<td>Pure Error</td>
<td>4.9369</td>
<td>4</td>
<td>1.2342</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cor Total</td>
<td>1264.546</td>
<td>16</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* $P = 0.05$, **$P = 0.01$

![Response contours for oil extractability.](image)
Table 5 shows the fatty acid of COSO using hexane as solvent and that extracted by AEP-SE. The composition of oleic acid and linoleic acid reached 77.76% and 8.34% respectively, and the fatty acid content did not depend on the extracted methods. The results show that the AEP-SE and OSE are both practical.

### 3.9. DPPH scavenging activity

The radical DPPH scavenging activities of oil recovered by AEP-SE and OSE were calculated as DPPH inhibition rates (%) (Fig. 8).

The results were compared with the data for commercially available standard ascorbic acid as

<table>
<thead>
<tr>
<th>Analytical characteristic</th>
<th>AEP-SE COSO</th>
<th>Hexane-extracted oil (Soxhlet)</th>
<th>Commercial COSO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Color (25.4 mm Cell)</td>
<td>Y35, R1.0</td>
<td>Y35, R6</td>
<td>Y35, R5</td>
</tr>
<tr>
<td>Free fatty acid (%)</td>
<td>0.05 ± 0.01</td>
<td>0.6 ± 0.02</td>
<td>1.5 ± 0.01</td>
</tr>
<tr>
<td>Peroxide value (mmol kg⁻¹)</td>
<td>0.5 ± 0.1</td>
<td>1.0 ± 0.1</td>
<td>6.0 ± 0.1</td>
</tr>
<tr>
<td>Moisture content (%)</td>
<td>0.08 ± 0.01</td>
<td>0.03 ± 0.01</td>
<td>0.05 ± 0.01</td>
</tr>
<tr>
<td>Iodine value (gI2/100 g)</td>
<td>84 ± 1</td>
<td>85 ± 2</td>
<td>89 ± 1</td>
</tr>
<tr>
<td>Saponification value (mg g⁻¹)</td>
<td>193 ± 2</td>
<td>195 ± 1</td>
<td>196 ± 2</td>
</tr>
</tbody>
</table>

Table 5

**Fatty acid composition of COSO with AE and OSE**

<table>
<thead>
<tr>
<th>Fatty acid composition (%)</th>
<th>AEP-SE</th>
<th>Hexane-extracted oil (Soxhlet)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Palmitic acid</td>
<td>9.75 ± 0.6</td>
<td>10.20 ± 0.5</td>
</tr>
<tr>
<td>Steatid acid</td>
<td>1.65 ± 0.05</td>
<td>1.70 ± 0.04</td>
</tr>
<tr>
<td>Oleic acid</td>
<td>77.76 ± 0.6</td>
<td>77.84 ± 0.5</td>
</tr>
<tr>
<td>Linoleic acid</td>
<td>8.34 ± 0.3</td>
<td>8.30 ± 0.4</td>
</tr>
<tr>
<td>Linolenic acid</td>
<td>0.36 ± 0.02</td>
<td>0.37 ± 0.01</td>
</tr>
<tr>
<td>Esosanoic acid</td>
<td>0.65 ± 0.0</td>
<td>0.59 ± 0.0</td>
</tr>
</tbody>
</table>

Figure 8

Antioxidant activities of COSO and OSE oil by DPPH radical scavenging assay.
positive control. All DPPH inhibition rates for the oil extracts, and standard ascorbic acid exhibited dose-dependent relationships. The aqueous extraction oil showed higher DPPH radical-scavenging activity than the organic solvent-extracted oil. Next, the concentration of samples required to scavenge 50% of DPPH radicals (IC50) was also calculated. The IC50 of standard ascorbic acid was 8.4 ± 0.17 mg mL⁻¹, whereas the values of oil recovered by AEP-SE and OSE were 2.27 ± 0.05 mg mL⁻¹ and 3.31 ± 0.07 mg mL⁻¹, respectively. A least significant difference test confirmed that the differences between the two methods were statistically significant (P < 0.05). AEP-SE derived oil exhibited higher antioxidant activity, which can be explained by its lower level of free fatty acid content and higher active compound content. These results are consistent with the data reported for aqueous system-extraction of sesame oil (Long et al., 2011; Latif et al., 2011; Ismail et al., 2010).

4. CONCLUSIONS

Aqueous extraction of COSO using SE was optimized. AEP-SE extracted 88.8 ± 1.4% of the total oil avoiding emulsification in the system, thereby resulting in lower processing costs. This method is an environmentally-friendly alternative technology to oil extraction with hexane that can be potentially applied in the oil industry.

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