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

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RESUMEN

Nuevo procedimiento para la extracción acuosa de aceite de semilla de *Camellia oleifera* y su actividad antioxidante

La extracción acuosa es una alternativa verde muy prometedora a la extracción con hexano. En este estudio se utilizó como ayuda en el proceso de extracción acuosa (AEP-SE) el efecto de una sal para la extracción de aceite de semilla de *Camellia oleifera* (COSO) para mejorar la extractabilidad y evitar la formación de emulsiones en el sistema acuoso. La mayor velocidad de extracción de aceite, 88,8%, se obtuvo con una concentración de carbonato de sodio de 1,48 mol L^{-1}, una relación de solución a la harina de 3,85, y 3.23h de tiempo de extracción, con una calidad del aceite extraído con el sistema acuoso similares a los de una muestra comercial de COSO extraida con hexano, en términos de índice de yodo, de color, y de índice de saponificación, aunque su contenido en humedad fue mayor. Además, el contenido de acidez libre del aceite extraido con el sistema acuoso fué menor que la del aceite extraído con disolvente. Los valores de la concentración inibihitoria al 50% del aceite obtenido por el AEP-SE y extracción con disolvente orgánico, medido por el ensayo de DPPH actividad de barrido, fueron 2,27 mg mL^{-1} y 3,31 mg mL^{-1}. Por lo tanto, AEP-SE es un método favorable al medio ambiente, prometedor para la preparación a gran escala de COSO.

PALABRAS CLAVE: Aceite de semilla – Actividad antioxidante – Camellia oleífera – Proceso acuoso.

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 saponifcation 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 inibihitory 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 *Camillia 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 CCl_4-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 the 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 (Hanmoungjai *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 degrade.

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.

Table 1
Composition of COS

Constituent	Kernel (g 100 g^{-1})
Moisture	7.5 ± 0.04
Crude oil	55.6 ± 0.02
Protein	15.8 ± 0.3
Ash	6.5 ± 0.8
Others (by difference)	14.6 ± 1.2

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

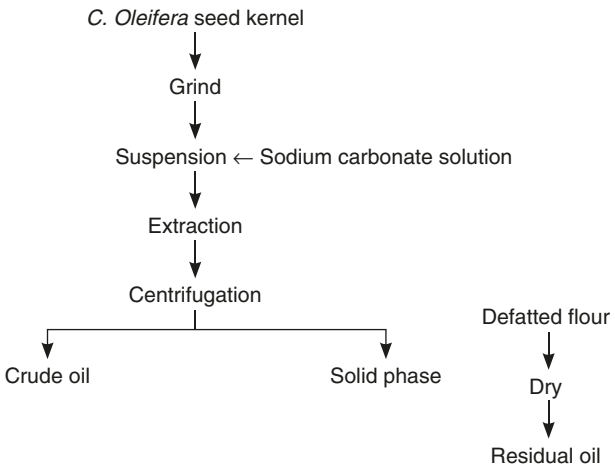

Figure 1
Key stages of the aqueous extraction of COSO.

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:

$$\text{Inhibition (\%)} = [(I_{Control} - I_{sample})]/(I_{control} - I_b) \times 100\%$$

where I_{sample}, $I_{control}$ 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% (IC_{50}), 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 Na_2CO_3 solutions (Fig. 2) suggesting that COSO can be extracted more effectively under salt effect (SE) conditions by Na_2CO_3. 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.

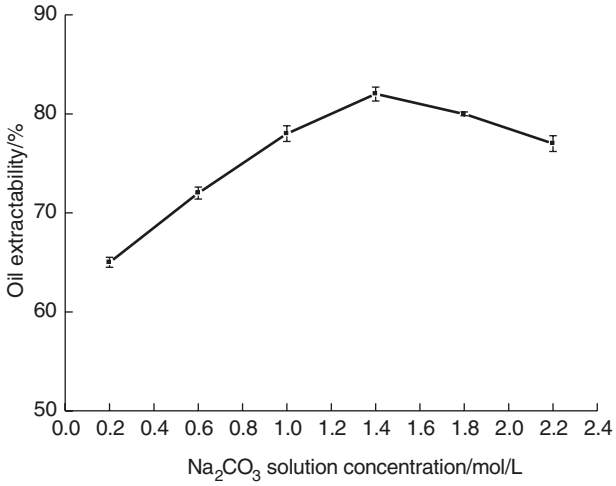

Figure 2
Effect of Na_2CO_3 solution concentrations on oil extractability.

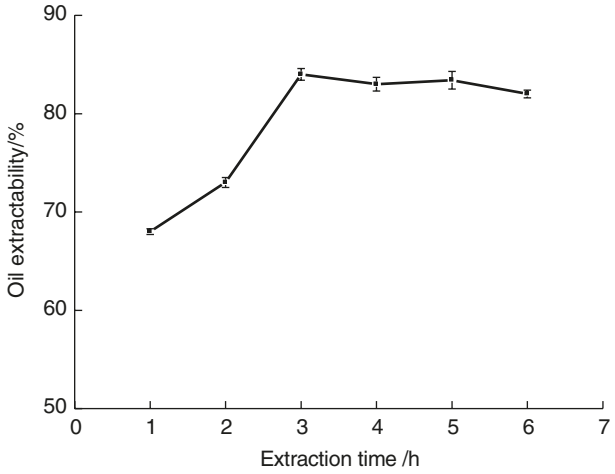

Figure 3
Effect of extraction time on oil extractability.

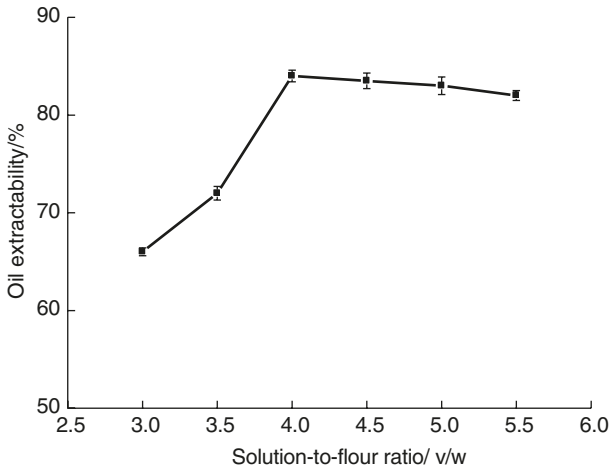

Figure 4
Effect of solution-to-flour ratio on oil extractability.

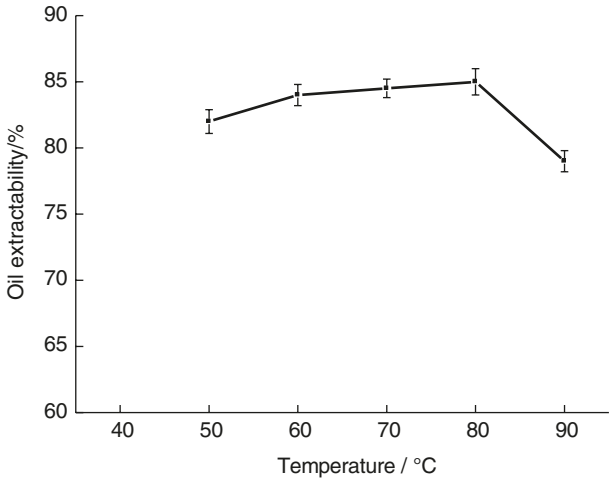

Figure 5
Effect of extraction temperature on oil extractability.

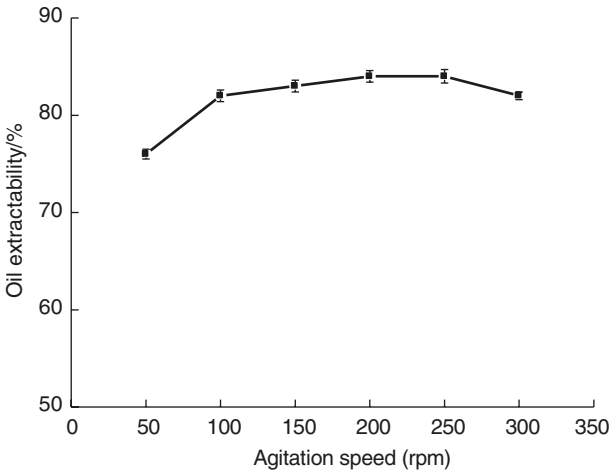

Figure 6
Effect of agitation speed on oil extractability.

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.

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.

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

Table 2
Experimental design and corresponding results for response surface analysis

Run	Coded variable (Actual variable)			Oil extractability Y/%
	Sodium carbonate concentration -X_1/mol/L	Solution-to-flour ratio-X_2	Extraction time-X_3h^{-1}	
1	–1(1.2)	–1(3)	0(3)	62.52
2	1(1.6)	–1	0	79.83
3	–1	1(5)	0	72.75
4	1	1	0	75.15
5	–1	0(4)	0	69.36
6	1	0	–1(2)	73.55
7	–1	0	1(4)	61.38
8	1	0	1	82.82
9	0(1.4)	–1	–1	72.65
10	0	1	–1	65.87
11	0	–1	1	81.43
12	0	1	1	75.35
13	0	0	0	86.71
14	0	0	0	88.11
15	0	0	0	87.41
16	0	0	0	87.81
17	0	0	0	85.32

[a] Values represent the means of two experiments.

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 time 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:

$$Y = 86.92 + 6.73X_1 - 0.91X_2 + 3.32X_3 - 3.73X_1X_2 + 2.56X_1X_3 - 9.36X_1^2 - 5.19X_2^2 - 7.73X_3^2 \quad (1)$$

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_2CO_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. These findings might be due to the fact that the extracted oil was dispersed into the system at increasing concentrations. 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

Table 3
ANOVA for the response surface quadratic models

Source	Sum of Squares	df	Mean Square	F Value	P-value (Prob > F)	Significant
Model	1180.9696	9	131.2188	10.9902	0.0023	**
X_1	304.31576	1	304.3157	25.4880	0.0015	**
X_2	6.67956	1	6.6795	0.5594	0.4789	
X_3	61.96136	1	61.9613	5.1895	0.0568	
X_1X_2	55.57706	1	55.5770	4.6548	0.0479	*
X_1X_3	14.22556	1	14.2255	1.1914	0.3112	
X_2X_3	0.1225	1	0.1225	0.01026	0.9222	
X_1^2	340.34546	1	340.3454	28.5057	0.0011	**
X_2^2	101.6042	1	101.6042	8.5099	0.0224	*
X_3^2	198.9888	1	198.9887	16.6663	0.0047	**
Residual	83.57678	7	11.9395			
Lack of Fit	78.6399	3	26.2132	21.2387	0.064	
Pure Error	4.9369	4	1.2342			
Cor Total	1264.546	16				

* P = 0.05, **P = 0.01

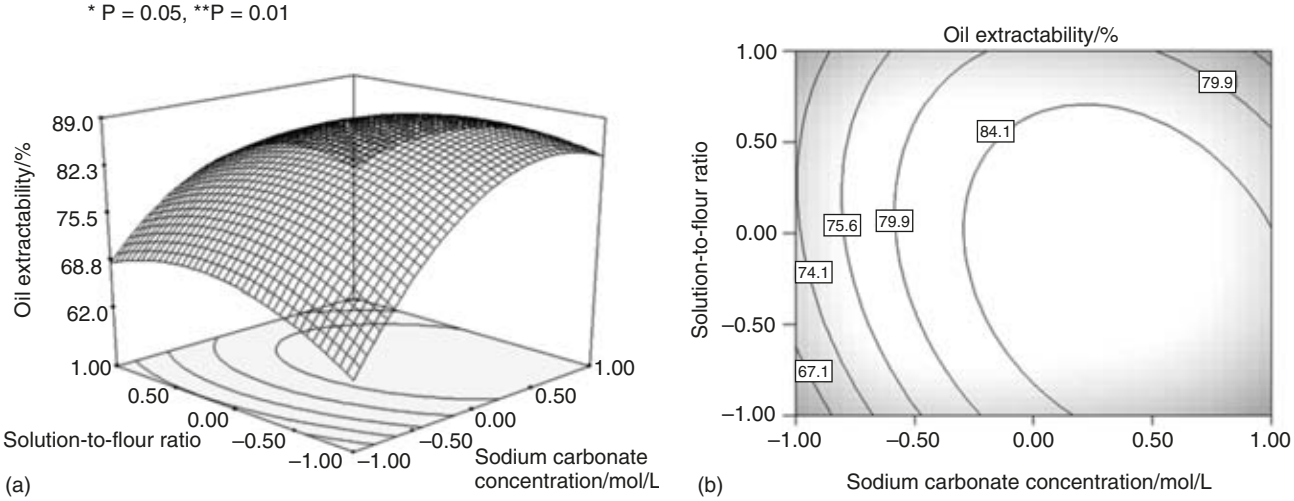

Figure 7
Response contours for oil extractability.

should be based on both factors. According to the response surface quadratic models, the optimal conditions for oil extractability are as follows: concentration of sodium carbonate, 1.48 mol L^{-1}, solution-to-flour ratio, 3.85, extraction time, 3.23 h. Under these conditions, the extractability can reach 88.8%.

3.7. Oil quality

The quality parameters for the oil obtained by AEP-SE are shown in Table 4, together with data for hexane-extracted oil and the commercial sample of COSO. The free fatty acid content, iodine value and saponification value of the oil obtained by aqueous extraction were clearly comparable with those of other samples.

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_2CO_3 during extraction.

3.8. Fatty acid composition of COSO

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

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 4
Comparisons between the qualities of COSO obtained by the different processes

Analytical characteristic	AEP-SE COSO	Hexane-extracted oil (Soxhlet)	Commercial COSO
Color (25. 4mm Cell)	Y35, R1.0	Y35, R6	Y35, R5
Free fatty acid (%)	0.05 ± 0.01	0.6 ± 0.02	1.5 ± 0.01
Peroxide value (mmol kg^{-1})	0.5 ± 0.1	1.0 ± 0.1	6.0 ± 0.1
Moisture content (%)	0.08 ± 0.01	0.03 ± 0.01	0.05 ± 0.01
Iodine value (gI_2/100 g)	84 ± 1	85 ± 2	89 ± 1
Saponification value (mg g^{-1})	193 ± 2	195 ± 1	196 ± 2

Table 5
Fatty acid composition of COSO with AE and OSE

Fatty acid composition (%)	AEP-SE	Hexane-extracted oil (Soxhlet)
Palmitic acid	9.75 ± 0.6	10.20 ± 0.5
Steatic acid	1.65 ± 0.05	1.70 ± 0.04
Oleic acid	77.76 ± 0.6	77.84 ± 0.5
Linoleic acid	8.34 ± 0.3	8.30 ± 0.4
Linolenic acid	0.36 ± 0.02	0.37 ± 0.01
Esosanoic acid	0.65 ± 0.0	0.59 ± 0.0

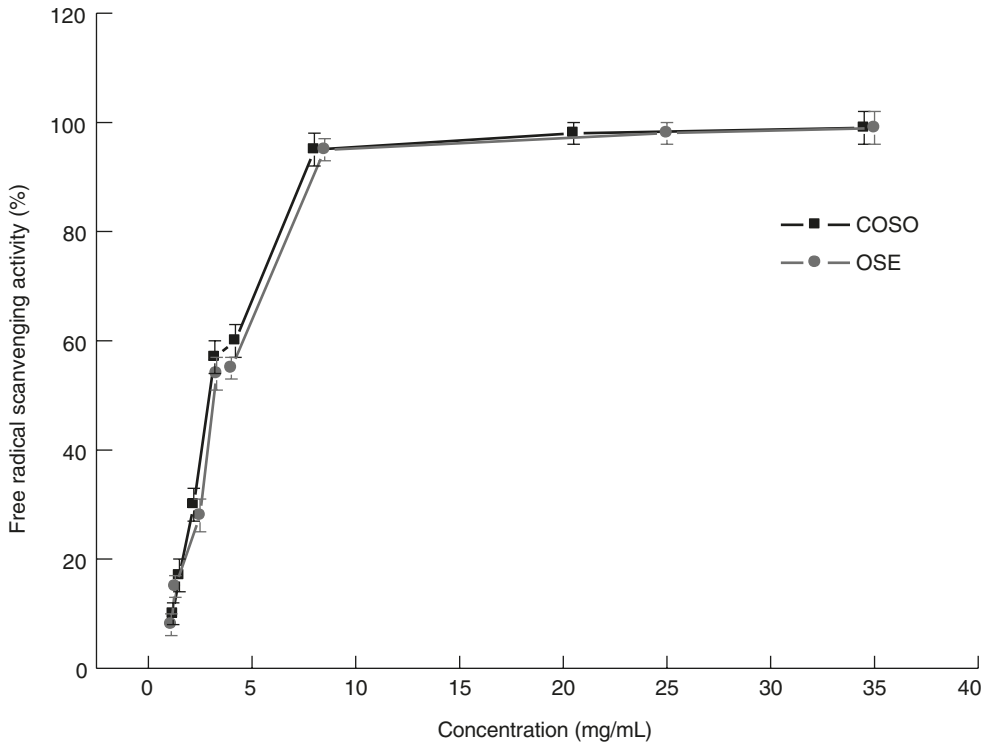

Figure 8
Antioxidant activities of COSO and OSE oil by DPPH radical scavenging assay.

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 (IC_{50}) was also calculated. The IC_{50} 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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