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Ultrasound-assisted aqueous enzymatic extraction of oil from perilla seeds and determination of its physicochemical properties, fatty acid composition and antioxidant activity

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Abstract

Response surface methodology (RSM) was used to optimize ultrasound-assisted aqueous enzymatic extraction (UAAEE) conditions for perilla seed oil. Under the optimum conditions—which were a liquid-to-solid ratio of 4.4:1, a hydrolysis time of 2.66 h, a hydrolysis temperature of 50.87 °C and an ultrasound treatment time of 24.74 min—an oil yield of 31.34% was obtained. Comparisons of the physicochemical characteristics of oil obtained using UAAEE with those of oil obtained by solvent extraction (SE oil) and cold pressing extraction (CPE oil) revealed similar refractive indices and saponification values, but UAAEE oil had a higher iodine value and better stability against oxidation, with a low peroxide value. UAAEE oil contained higher levels of beneficial α-linolenic acid and phenolics than SE oil or CPE oil, and it also had superior efficiency in scavenging 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals.

Keywords: perilla seed oil; ultrasound-assisted aqueous enzymatic extraction; physical and chemical characteristics; fatty acid composition; antioxidant activities.

Practical Application: Based on its physical and chemical properties, perilla oil obtained using UAAEE would be suitable as high quality edible oil and could also have applications in the cosmetic and pharmaceutical industries.

1 Introduction

Perilla (*Perilla frutescens*) is an annual herb that is widely used for cooking and medicinal purposes in several Asian countries (Igarashi & Miyazaki, 2013), including China, Japan and Korea. Perilla seed oil is rich in omega-3 fatty acids (53.6%-64%), especially α-linolenic acid (ALA, 18:3, n-3, 52.58%-61.98%) (Zhou et al., 2014). ALA is metabolized to long-chain polyunsaturated fatty acids (LC-PUFA) such as eicosapentaenoic acid (EPA, 20:5, n-3) and docosahexaenoic acid (DHA, 22:6, n-3), which are especially important during human brain development (Lee & Song, 2012). Because of its high omega-3 fatty acid content, perilla seed oil also has the potential to lower the risk of many chronic diseases. It may reduce inflammation, prevent abnormal clotting, relax blood vessels, and have anticancer properties (Asif, 2011; Li et al., 2014).

Perilla seed oil is typically obtained either by mechanical pressing or by extraction using organic solvents. However, the yield obtained by mechanical pressing is quite low and the use of organic solvents causes unacceptable contamination of the environment and residues in the oil may be harmful to human health (Zhang et al., 2010). Aqueous enzymatic extraction (AEE) has been successfully used for the extraction of oils from a variety of oil-bearing seeds, including olive (Najafian et al., 2009) and peanut (Jiang et al., 2010). Ultrasound has been shown to accelerate heat and mass transfer and is a powerful alternative to conventional extraction techniques. The strong mass transmission effect, which is mainly caused by cavitational effects (Chemat et al., 2011), could be used to enhance the extraction efficiency of AEE for edible oils. Ultrasound-assisted extraction (UAE) has many advantages, including high extraction yields, high reproducibility, low solvent use, short extraction times, low running costs, limited environment impact and easy adaptation to industrial scale use (Vuong et al., 2014).

In the present study, optimal conditions for ultrasound-assisted aqueous enzymatic extraction (UAAEE) of oil from perilla seeds were investigated using response surface methodology (RSM). The fatty acid composition, physicochemical properties and antioxidant activities of perilla seed oil extracted by UAAEE were evaluated and compared with those of mechanically pressed oil and oil extracted using organic solvent.

2 Materials and methods

2.1 Plant materials and reagents

P. frutescens seeds (oil content 37.8%) were collected in October 2014 from the North University of China (Taiyuan, Shanxi Province, China). The dried seeds were milled to approximately 250 μm and stored in airtight containers at 4 °C until use. Chemical reagents were supplied by Beijing Chemical Reagents Co. (Beijing, China).

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2.2 Soxhlet extraction (SE) and cold pressing extraction (CPE)

Perilla seeds (dried and milled, 5 g) were extracted with *n*-hexane (250 mL) in a Soxhlet extractor at 90 °C for 7 h. The *n*-hexane was removed at 50 °C under reduced pressure using a rotary evaporator (SHZ-95B, Yuhua Ltd., Gongyi, Henan, China). SE gave an extraction yield of 35.2% (w/w). An oil sample was also prepared by CPE. The oil samples were stored in glass vials at 4 °C prior to physical and chemical characterization.

2.3 Ultrasound-assisted aqueous enzymatic extraction (UAAEE)

Powdered perilla seeds (15 g) were pretreated with water (water/seed ratio, 3:1 to 7:1 mL/g) in a tunable ultrasonic bath (TH-400BQG, 50Hz; Tianhua Ultrasonic Electronic Equipment Co., Ltd., Jining, Shandong, China) at 30 °C for 20 min-30 min at a power of 400 W. An enzyme cocktail (cellulase (5.5%) / neutral proteinase (4.5%) / pectinase (7.5%)) was then added and the mixture was incubated in a water bath at 45 °C-55 °C for 2 h-3 h with constant stirring. Before adding the enzymes, the pH of the mixture was adjusted to 7.0 using 0.5 M NaOH and 0.5 M HCl. After the extraction process, the mixture was centrifuged at 8000 g for 20 min at room temperature, giving three layers (oil, cream and skim). The uppermost oil layer was carefully removed using a micropipette. The oil yield per seed sample (100 g), on a dry-weight basis, was determined using the Equation 1:

$$Oil\ yield\ (\%) = (weight\ of\ extracted\ oil / weight\ of\ seeds) \times 100\% \quad (1)$$

2.4 RSM design and statistical analysis

Based on the results of a preliminary mono-factor test (data not shown), a Box-Behnken design (BBD) was used to determine the effects of four independent variables, i.e., liquid/solid ratio (X_1), hydrolysis time (X_2), hydrolysis temperature (X_3) and ultrasound treatment time (X_4), on perilla oil yield (Y). The independent variables were coded at three levels (–1, 0, and 1). The complete design consisted of 29 experimental points, including five replicates of the central points (all variables were coded as zero). A randomized experimental order was used to reduce the effect of unexplained variability on the observed responses. The run order, variable conditions, and experimental and predicted values are shown in Table 1. A regression analysis was carried

Table 1. Box-Behnken design and observed responses of perilla oil yield using UAAEE.

Run	Liquid-solid ratio X_1 (mL:g)	Hydrolysis ttime X_2 (h)	Hydrolysis temperature X_3 (°C)	Ultrasonic time X_4 (min)	Oil yield (%)	
					Experimental values	predicted values
1	5:1	2.0	50	30	27.53	26.67
2	5:1	2.5	55	30	28.33	28.42
3	7:1	2.5	50	20	25.13	25.32
4	7:1	2.0	50	25	24.67	24.48
5	7:1	2.5	45	25	25.67	25.74
6	5:1	3.0	45	25	28.53	28.03
7	5:1	3.0	50	30	27.93	27.28
8	5:1	2.0	55	25	26.07	26.36
9	5:1	3.0	50	20	28.13	28.75
10	3:1	2.5	45	25	28.47	28.42
11	5:1	2.5	50	25	31.33	30.96
12	5:1	2.5	50	25	31.27	30.96
13	5:1	2.5	50	25	29.33	30.96
14	5:1	2.5	50	25	31.40	30.96
15	5:1	2.5	45	20	27.73	28.09
16	5:1	2.0	45	25	27.93	27.09
17	3:1	2.0	50	25	24.47	25.66
18	7:1	2.5	50	30	24.13	24.99
19	5:1	3.0	55	25	28.87	29.50
20	5:1	2.5	45	30	26.93	27.89
21	5:1	2.5	55	20	28.80	28.29
22	3:1	3.0	50	25	28.60	29.23
23	3:1	2.5	50	30	28.40	28.00
24	7:1	3.0	50	25	25.73	24.99
25	5:1	2.5	50	25	31.47	30.96
26	7:1	2.5	55	25	26.27	26.08
27	3:1	2.5	55	25	29.13	28.82
28	5:1	2.0	50	20	24.87	25.28
29	3:1	2.5	50	20	28.80	27.74

out to evaluate the response function as a quadratic polynomial (Equation 2):

$$Y = \beta_0 + \sum_{i=1}^{4} \beta_i X_i + \sum_{i=1}^{4} \beta_{ii} X_i^2 + \sum_{i=1}^{3} \sum_{j=i+1}^{4} \beta_{ij} X_i X_j \quad (2)$$

where Y is the response variable (oil yield); β_0 is the offset term, β_i is the linear effect, β_{ii} is the squared effect, β_{ij} is the interaction effect and $X_{i,}$ and X_j are independent variables.

Data were analyzed by one-way analysis of variance (ANOVA) to determine the lack of fit and the effects of linear, quadratic, and interaction variables on perilla oil extraction yields. Data analyses and RSM were performed using Design-Expert® Software version 8 (Stat-Ease, Inc., Minneapolis, MN, USA).

2.5 Characterization of perilla oil

Refractive indices were determined using a Series RA-130 refractometer (KEM-China, Shanghai, China). ISO (International Organization for Standardization) standard methods were used for the determination of peroxide value, acid value, iodine value, saponification value, and unsaponifiable matter.

2.6 Fatty acid composition

The fatty acid composition of the perilla seed oils was analyzed using an HP-7900 gas chromatograph (Tianmei Scientific Instrument Co., Ltd., Shanghai, China), according to the method of Li et al. (2015).

2.7 Determination of total tocopherols (TT) and total phenolics (TP)

The TT contents of perilla seed oils were determined using the colorimetric method described by Wong et al. (1988). The TP contents were determined using the Folin-Ciocalteu method (Capannesi et al., 2000).

2.8 DPPH scavenging activity

The DPPH radical-scavenging activity of perilla seed oil extracted using UAAEE was determined using the method described by Long et al. (2011).

3 Results and discussion

3.1 Fitting the mathematical model

The ANOVA results for the quadratic model are shown in Table 2. The model *F* value (9.14) and low *P* value ($P < 0.0001$) showed that the model was statistically significant. A model is considered to be adequate when the coefficient of determination (R^2) is > 0.75. In this case, $R^2 = 0.9014$ (indicating that 90.14% of the experimental oil yield values matched the model-predicted values) and is sufficient to validate the significance of the model. Furthermore, lack of fit, which measures the fitness of the model, was not significant ($P > 0.05$). Based on these factors, the mathematical model was considered to be adequate for the prediction of oil yield and was fitted to the following second-order polynomial equation, $Y = 30.960 - 1.356X_1 + 1.021X_2 + 0.184X_3 - 0.018X_4 - 0.768X_1X_2 - 0.015X_1X_3 - 0.150X_1X_4 + 0.550X_2X_3 - 0.715X_2X_4 + 0.083X_3X_4 - 2.676X_1^2 - 2.194X_2^2 - 1.019X_3^2 - 1.771X_4^2$.

Table 2. Analysis of variance (ANOVA) for the quadratic polynomial mode.

source	Sum of squares	df	Mean square	*F* value	*P*-value[a] Prob *F*
Model	113.25	14	8.09	9.14	< 0.0001
X_1	22.06	1	22.06	24.92	0.0002
X_2	12.51	1	12.51	14.13	0.0021
X_3	0.41	1	0.41	0.46	0.5088
X_4	0.004	1	0.004	0.004	0.9495
$X_1 X_2$	2.36	1	2.36	2.66	0.1251
$X_1 X_3$	0.0009	1	0.0009	0.001	0.9750
$X_1 X_4$	0.09	1	0.09	0.10	0.7546
$X_2 X_3$	1.21	1	1.21	1.37	0.2619
$X_2 X_4$	2.04	1	2.04	2.31	0.1508
$X_3 X_4$	0.03	1	0.03	0.03	0.8633
X_1^2	46.46	1	46.46	52.48	< 0.0001
X_2^2	31.22	1	31.22	35.26	< 0.0001
X_3^2	6.73	1	6.73	7.60	0.0154
X_4^2	20.35	1	20.35	22.99	0.0003
Residual	12.39	14	0.89		
Lack of fit	9.05	10	0.91	1.08	0.5123
Pure error	3.34	4	0.84		
Cor total	125.65	28			
R^2	0.9014				
Adj.R^2	0.8027				

[a]$P<0.01$ highly significant; $0.01<P<0.05$ significant; $P>0.05$ not significant.

The effects of X_1, X_2, X_1^2, X_2^2 and X_4^2 on oil yield were highly significant ($P < 0.01$). Similarly, the effect of X_3^2 was significant ($P < 0.05$). On the other hand, X_3, X_4, X_1X_2, X_1X_3, X_1X_4, X_2X_3, X_2X_4 and X_3X_4 had no significant effects ($P > 0.05$) on oil yield. Based on the linear and quadratic coefficients, we concluded that the order of importance of different variables on oil yield was: liquid-to-solid ratio > hydrolysis time > hydrolysis temperature > ultrasound treatment time.

3.2 RSM analysis

The best way to visualize the effect of independent variables on a dependent variable is to draw three-dimensional (3D) response surface curves of the model. The 3D plot of the response surface for the oil yield against the liquid-to-solid ratio and hydrolysis time shows that a moderate liquid-to-solid ratio resulted in a high extraction yield (Figure 1a). As the hydrolysis time increased, the oil yield increased until it reached a plateau at > 2. 66 h and subsequently decreased slightly.

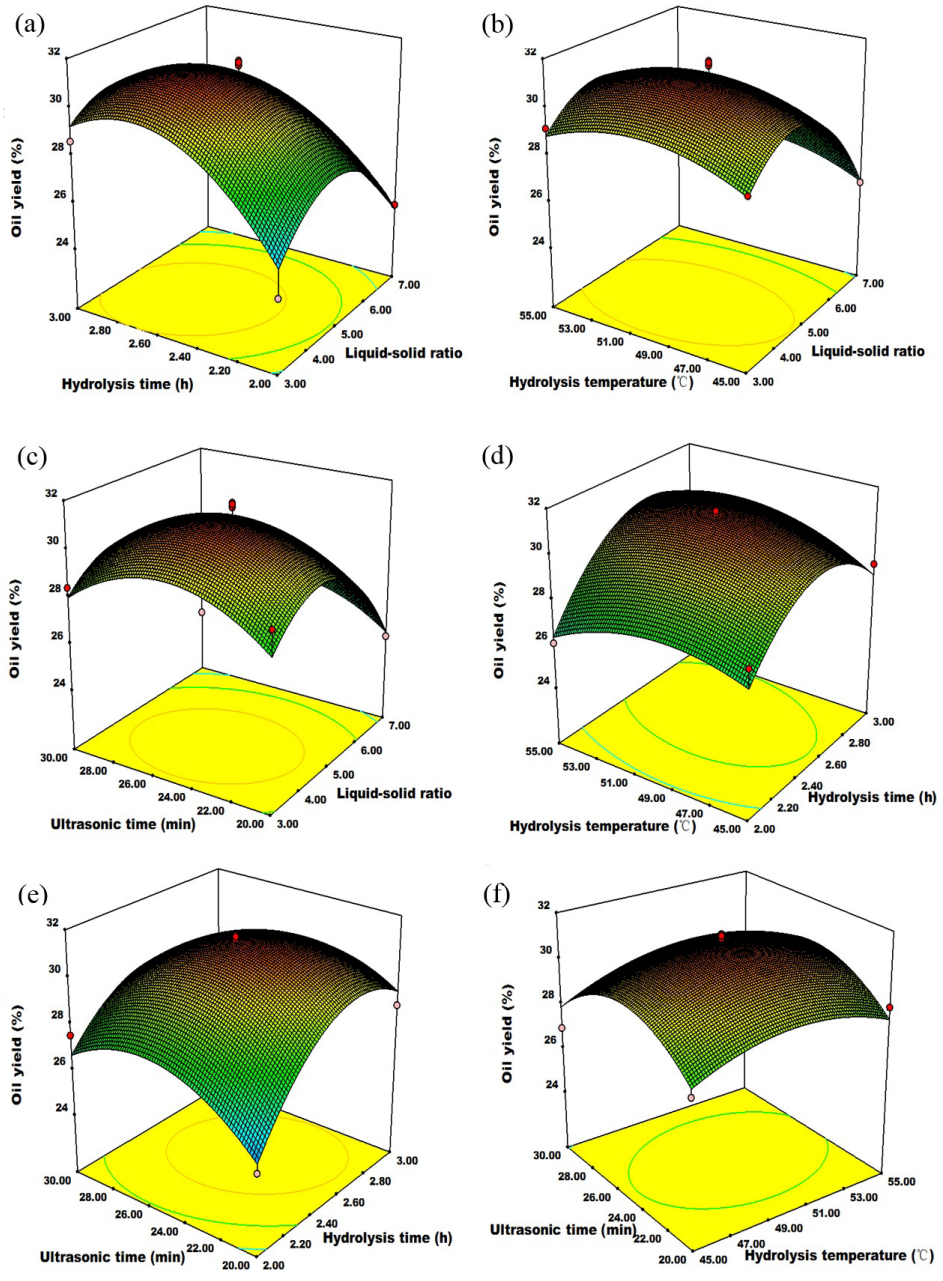

Figure 1. Response surface plots showing correlation of (a) liquid-solid ratio and hydrolysis time; (b) liquid-solid ratio and hydrolysis temperature; (c) liquid-solid ratio and ultrasonic time; (d) hydrolysis time and hydrolysis temperature; (e) hydrolysis time and ultrasonic time, and (f) hydrolysis temperature and ultrasonic time on the oil yield obtained using Ultrasound-assisted aqueous enzymatic extraction (UAAEE).

A medium liquid-to-solid ratio and long hydrolysis time were favorable for extracting perilla oil (Figure 1b and Figure 1d). The influence of the independent variable, hydrolysis temperature, was not as significant as those of the liquid-to-solid ratio and hydrolysis time. The oil yield increased with increasing temperature up to 50.87 °C. Similar effects on the extraction yield were seen in the curves for liquid-to-solid ratio and ultrasound treatment time (Figure 1c), and hydrolysis time and ultrasound treatment time (Figure 1e).

The best oil yields were obtained with hydrolysis temperatures of 50.87 °C and ultrasound treatment times of 24.74 min (Figure 1f). It is likely that increases in hydrolysis temperature and ultrasound treatment time shorten the time of mass transfer and improve oil extraction rates.

3.3 Verification of the predictive model

According to the second-order polynomial equation, the optimum conditions for oil yield were: liquid-to-solid ratio, 4.4:1; hydrolysis time, 2.66 h; hydrolysis temperature, 50.87 °C; and ultrasound treatment time, 24.74 min. Under these conditions, the predicted oil yield was 31.34%, which was lower than that obtained by SE (35.2%) but higher than that obtained by CPE (28.6%). To further validate the reliability of the theoretical model prediction, verification experiments ($n = 3$) were carried out under optimal conditions. The experimental value (31.28%) was found to be consistent with the predicted value. Therefore, the oil extraction conditions obtained using RSM were not only accurate and reliable, but also had practical value (Xu et al., 2013).

3.4 Physicochemical characteristics and fatty acid composition of perilla oil

The physicochemical characteristics of the perilla oils obtained by UAAEE, SE and CPE are shown in Table 3. At room temperature, all oil samples were yellow and there were no significant differences ($p > 0.05$) in their refractive indices (1.479 - 1.481). The iodine values of UAAEE oil (210.654 g/100 g oil) and SE oil (213.192 g/100 g oil) were significantly higher ($p < 0.05$) than that of CPE oil (196.695 g/100 g oil), which suggests that UAAEE oil and SE oil have higher levels of unsaturated fatty acids (Table 3). However, the acid value of UAAEE oil (3.058 mg/g oil) was significantly higher ($p < 0.05$) than that of SE oil (1.299 mg/g oil) or CPE oil (1.143 mg/g oil). This difference may be caused by the action of endogenous lipases, which were not immediately inactivated during the extraction process. The peroxide value of UAAEE oil (3.2 meq. O_2/kg oil) was also significantly lower ($p < 0.05$) than that of CPE oil (4.3 meq. O_2/kg oil) or SE oil (4.5 meq. O_2/kg oil). The higher peroxide value of SE oil is probably attributable to accelerated oxidation brought about by the elevated operational temperature and prolonged extraction time used in the conventional SE process (Latif & Anwar, 2011). The saponification values (196.48 - 198.26 mg KOH/g oil) of UAAEE oil, SE oil and CPE oil were not significantly different ($p > 0.05$). The values were, however, higher than those of other vegetable oils (Cerchiara et al., 2010; Li et al., 2015), indicating very high levels of low molecular weight triacylglycerols, which means that perilla oil is very suitable for the production of liquid soaps and shampoos.

The fatty acid composition of perilla oil is also shown in Table 3. Palmitic acid ($C_{16:0}$, 4.43%-5.62%) and stearic acid ($C_{18:0}$, 1.86%-1.92%) were the principal saturated fatty acids

Table 3. Physicochemical characteristics and fatty acid composition of perilla oil.

	UAAEEO	SEO	CPEO
Physicochemical characteristics			
Refractive index	1.480 ± 0.001a	1.481 ± 0.002a	1.479 ± 0.001a
Acid value (mg/g oil)	3.058 ± 0.03a	1.299 ± 0.03b	1.143 ± 0.02c
Iodine value (g/100 g oil)	210.654 ± 0.35a	213.192 ± 0.50a	196.695 ± 0.14b
Saponification value (mg KOH/g oil)	196.48 ± 0.47a	198.26 ± 0.35a	197.82 ± 0.45a
Peroxide value (meq. O_2 / kg oil)	3.2 ± 0.03b	4.5 ± 0.05a	4.3 ± 0.03a
Total tocopherol (mg/kg)	450.88 ± 1.35b	490.10 ± 1.20a	383.25 ± 1.25c
Total phenolics (mg GAE/kg)	615.25 ± 0.66a	410.50 ± 0.45b	343.12 ± 0.30c
Physical state at room temperature	Liquid	Liquid	Liquid
Fatty acid composition (%)			
Palmitic acid ($C_{16:0}$)	4.43 ± 0.05c	4.70 ± 0.02b	5.62 ± 0.01a
Stearic acid ($C_{18:0}$)	1.87 ± 0.01a	1.86 ± 0.01a	1.92 ± 0.02a
Oleic acid ($C_{18:1}$)	20.39 ± 0.15b	16.92 ± 0.20c	21.16 ± 0.10a
Linoleic acid ($C_{18:2}$)	9.12 ± 0.08c	14.53 ± 0.15a	11.19 ± 0.05b
Linolenic acid ($C_{18:3}$)	64.05 ± 0.20a	62.26 ± 0.35b	60.11 ± 0.15c
SAFA	6.30c	6.56b	7.54a
MUFA	20.39b	16.92c	21.16a
PUFA	73.17b	76.79a	71.3c
TUFA	93.56a	93.71a	92.46b
P/S	11.61a	11.71a	9.46b

Mean values ± standard deviation (n=3) followed by different letters differ at $p < 0.05$, according to Duncan (SSR) test. SAFA: Saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; TUFA: total unsaturated fatty acids.

(SFA); oleic acid ($C_{18:1}$, 16.92%-21.16%) was the predominant monounsaturated fatty acid (MUFA); and linoleic acid ($C_{18:2}$, 9.12%-13.53%) and ALA ($C_{18:3}$, 60.11%-64.05%) were the two main polyunsaturated fatty acids (PUFA). Perilla seed oil is very rich in MUFA and PUFA, which account for > 90% of the total fatty acids, and contains relatively low amounts of SFA. Unsaturated fatty acids can affect the physical properties of cell membranes, including fluidity and permeability (Nasri et al., 2005). The omega-3 fatty acid ALA is a precursor of EPA and DHA, which are especially beneficial to human health (Lee & Song, 2012). We have shown that the ALA content of UAAEE oil (64.05%) is significantly higher ($p < 0.05$) than that of SE oil (62.26%) or CPE oil (60.11%). The oleic acid content of UAAEE oil (20.39%) is also significantly higher ($p < 0.05$) than that of SE oil (16.92%). Oleic acid is very important in the construction of nerve cells and has a fundamental role in the prevention of cardiovascular diseases (Nasri et al., 2005). The health benefits of perilla seed oil obtained by UAAEE were thus greater than those of oil obtained by conventional SE or CPE.

Perilla seed oil has a polyunsaturated/saturated (P/S) ratio in the range 9.46-11.71, which is demonstrated by its high refractive index. The P/S ratio of UAAEE oil (11.61) is not significantly different ($p > 0.05$) from that of SE oil (11.71), but is significantly higher ($p < 0.05$) than that of CPE oil (9.46). A high P/S ratio is associated with reduction of serum cholesterol levels and atherosclerosis risk and with the prevention of heart disease (Oomah et al., 2002).

3.5 TT and TP content

Tocopherols and phenolics are important antioxidants found in plant oils that act as chain-breakers in free radical chain reactions and convert lipid radicals into more stable products (Gai et al., 2013). We found that the TT content of SE oil (490.10 mg/kg) was significantly higher ($p < 0.05$) than that of UAAEE oil (450.88 mg/kg) or CPE oil (383.25 mg/kg). High amounts of tocopherols are typically associated with high PUFA content (Tuberoso et al., 2007) and we found a significant positive correlation between PUFA and TT content ($r = 0.944$). TP content in UAAEE oil (615.25 mg GAE/kg) was, however, significantly higher ($p < 0.05$) than in SE oil (410.50 mg GAE/kg) or CPE oil (343.12 mg GAE/kg). TP content in UAAEE oil may be higher because enzymatic hydrolysis reduces the complexation of phenolic compounds with seed polysaccharides, proteins and pectins, thereby enhancing their partitioning into the oil phase (Ranalli et al., 2005). It is also noteworthy that TT content in perilla oil is higher (383.88-490.10 mg/kg) than in peanut oil (165.5 mg/kg) or rapeseed oil (151.5 mg/kg). TP content in perilla oil (343.12-615.25 mg GAE/kg) is higher than that in *Albizia julibrissin* oil (35.43 mg GAE/kg), soybean oil (173.67 mg GAE/kg) or sesame seed oil (24.00 mg GAE/kg) (Nehdi, 2011; Latif & Anwar, 2011). Perilla oil is thus a good source of the natural antioxidants, tocopherols and phenolics.

3.6 Antioxidant activities

As well as protecting the human body from diseases such as cancer, inflammation, rheumatoid arthritis and cardiovascular disorders (Tang et al., 2014), antioxidants are also able to prevent food spoilage. Recently, interest in the antioxidant properties of plant seed oils and their benefits to human health has increased considerably.

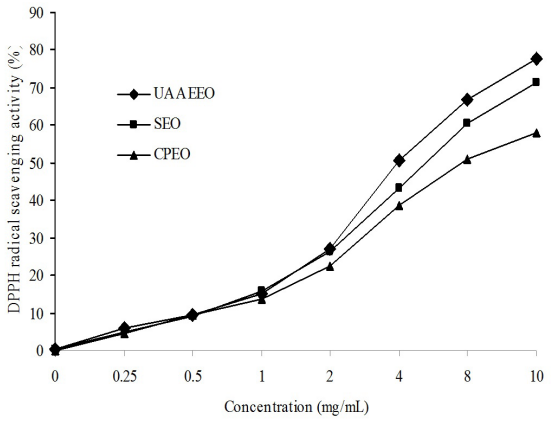

Figure 2. Antioxidant activities of the oils assessed by DPPH radical-scavenging assay.

DPPH radical-scavenging assays can be used as rapid and reliable tests for predicting the oxidative stability of fatty food such as oils, margarines and meat products (Gai et al., 2013). DPPH radical-scavenging activity was shown to increase with increasing concentration of perilla seed oils (Figure 2) UAAEE oil had a significantly ($p < 0.05$) lower IC_{50} value (3.99 mg/mL) than SE oil (4.80 mg/mL) or CPE oil (7.19 mg/mL), indicating that UAAEE oil has the best DPPH radical-scavenging activity. Previous studies have demonstrated that the antioxidant potential of plant oils can be attributed mainly to PUFA, tocopherols and phenolics (Gai et al., 2013). In the present study, UAAEE oil showed higher antioxidant activity than SE oil or CPE oil, likely because of the higher phenolic content of UAAEE oil (615.25 mg GAE/kg) compared with SE oil (410.50 mg GAE/kg) or CPE oil (343.12 mg GAE/kg). A significant ($p < 0.05$) negative correlation was seen between TP content and IC_{50} values ($r = -0.846$).

4 Conclusion

In this study, a UAAEE method for perilla seed oil was optimized using RSM and an efficient, high performance, process was achieved. The optimum UAAEE conditions for perilla oil were: enzyme cocktail, cellulase (5.5%) / neutral proteinase (4.5%) / pectinase (7.5%); liquid-to-solid ratio, 4.4:1; hydrolysis time, 2.66 h; hydrolysis temperature, 50.87°C; and ultrasound treatment time, 24.74 min. Under these conditions, the predicted maximum oil yield was 31.34%, which was lower than that obtained using SE (35.2%) and higher than that obtained using CPE (28.6%). The predicted oil yield value agreed well with the experimental value. Comparisons of the physicochemical characteristics of UAAEE oil with those of SE oil and CPE oil revealed similar refractive indices and saponification values, but UAAEE oil had a higher iodine value and better stability against oxidation, with a low peroxide value. Additionally, UAAEE oil contained high levels of beneficial ALA, which were significantly ($p < 0.05$) higher than those of SE oil or CPE

oil. UAAEE oil was also more effective in scavenging DPPH radicals. The lower IC_{50} value compared with those of SE oil and CPE oil could be partly explained by the higher levels of phenolics in UAAEE oil (615.25 mg GAE/kg) compared with SE oil (410.50 mg GAE/kg) and CPE oil (343.12 mg GAE/kg). Overall, UAAEE is a promising and environmentally-friendly technique for oil extraction in the food industry. The present investigations indicate that perilla oil obtained using UAAEE is suitable for use as high quality edible oil and could also have applications in the cosmetic and food industries.

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