Optimization of ultrasound-assisted-extraction of porcine placenta water-soluble proteins and evaluation of the antioxidant activity

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Abstract Porcine placenta is commonly used in Chinese as a traditional medicine. It has been reported by a number of researchers that the porcine placenta contains many compounds which have good health benefits. Response surface methodology (RSM) was applied to optimize the parameters of ultrasound power, ultrasound treat time, and extraction temperature on the extraction yield of porcine placenta water-soluble proteins (PPWP). The results indicated that, under optimum conditions of ultrasound power 257 w, extraction temperature at 49 °C for 7 min, the extraction yield of PPWP was 32.7 %, which was significantly higher than that of the conventional extraction method (CEM) of 15.0 %. The experimental data were fitted to a second-order polynomial equation using multiple regression analysis and the estimated model showed a high capacity of predicting the dependent variables. Although not significantly, the antioxidant activity of PPWP from ultrasound-assist-extraction (UAE) were higher than those from CEM, indicated that UAE had a positive effect or at least no negative effect on the bioactivity of PPWP.

Keywords Porcine placenta · Protein · Ultrasound · Extraction · Antioxidant activity

Introduction

Porcine placenta is commonly used in Chinese traditional medicine (Chen et al. 1999; Zhang et al. 2007). It has been reported that the porcine placenta contains many bioactive compounds that may have health benefits. Georgieva et al. (1995) reported the potential immunostimulatory and immunosuppressive activities of pig fetal placenta extracts. Good immunobiologic and anti-aging activities were also observed in pig placenta polypeptide extract (Chen et al. 1999). Experiments from Lee et al. (2012) indicated that porcine placenta steroid extract was an effective inducer of lipid accumulation and transdifferentiation with no cell toxicity. Furthermore, Jash et al. (2011) investigated the effect of porcine placenta extract on contact hypersensitivity, and the results suggested that porcine placenta extract have a therapeutic potential to modulate skin inflammation. It is noted that the bioactive compounds responsible to the above studies were proteins and/or peptides.

Traditional methods of extracting proteins/peptides from porcine placenta were “freeze-melting” and solvent extraction (Jash et al. 2011; Wang et al. 2011). For freeze-melting method, the porcine placenta is frozen to form ice crystals between the cells, and then thawed at 37 °C. The cell walls are broken in this process, and cellular materials (proteins/peptides, minerals, vitamins etc.) are leaked out. Nevertheless, the freeze-melting process is time consuming and inefficient, which implies this method may only be used in lab scale. Comparatively, the solvent extraction method, with utilization of physiological saline, phosphate buffer solution (PBS) and deionized water (Fang 2007), is more efficient and used as a common method in past decades. However, all these conventional extraction methods have a low extraction yield.

The more widely used methods for extraction of protein might be mechanical and chemical extraction, using alkaline, salt and ethanol as solvents and stirring the mixture at a certain

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temperature. However, mechanical breakage of cell wall of the extracting material is also labor-intensive and time-consuming while large amounts of samples are required, and chemical extractions may introduce interfering compounds and result in purification difficulty (Ge et al. 2010). On the other hand, some extraction solvents may cause denaturation of the protein, decrease the bioactivity and reduce nutritive values (Zhu et al. 2009; Jodayree et al. 2012). A number of new technologies have been reported for extract of proteins, such as ultrasound-assist-extraction (UAE), microwave-assist-extraction (MAE), ionic liquid solution extraction (Ge et al. 2010), high density steam flash-explosion (Zhang et al. 2013) and fermentation extraction (Schindler et al. 2011), where the UAE and MAE are commonly used to increase the extraction efficiency and reduce solvent usage. The technique of UAE can enhance the mass transfer through acoustic-induced cavitation and has been successively used in extraction of a variety of bioactives (Chemat et al. 2011). When mechanical waves of ultrasound are transmitted through a fluid, the average distance within molecules is modified, oscillating around their equilibrium position. The distance of intermolecular been shorten and lengthen during the compression cycle and rarefaction cycle, respectively. In the rarefaction cycle, the pressure decrease enough to exceed the critical distance between molecules, then cavities and cavitation bubbles can appear in the bulk liquid. Those cavitation bubbles could produce microjet and shockwave in the solid–liquid interface of a heterogeneous medium, so enhance the mass transfer of intracellular content and external solvent in solid/liquid system (Esclapez et al. 2011). UAE also shows a good extraction yield and can be applied in the extraction of heat-sensitive compounds with minimal damage (Kadam et al. 2013). Although a number of bioactive compounds have been extracted from plant materials using UAE, such as isoflavones from *Radix Puerariae* (Lee and Lin 2007), baicalin and baicalein from *Radix Scutellariae* (Yang et al. 2013), polysaccharide-protein complexes from mushrooms (Cheung et al. 2012), polyphenols from apple pomace (Pingret et al. 2012), and flavonoid and polyphenols and methylxanthines from yellow tea (Horžić et al. 2012) etc., there is limited investigation of UAE on animal materials (Vilkhu et al. 2008). It was reported that extraction of lutein from egg yolk using UAE was efficient with minimal bioactivity degradation (Yue et al. 2006), which suggested that UAE may be applied in extracting bioactives from animal materials. The purpose of this work was to using UAE to increase the extraction yield of porcine placenta water-soluble proteins (PPWP). Response surface methodology (RSM) was employed to optimize the UAE conditions. Furthermore, the antioxidant activity of PPWP was also evaluated by using the DPPH (1,1-diphenyl-2-picrylhydrazyl) radical, superoxide anion radical and hydroxyl radical assays.

Materials & methods

Materials and chemicals

Porcine placenta was obtained by courtesy of JiaHao Company (Zhongshan, Guangdong province, China). After arrival, the samples were washed with tap water until no blood or impurities were observed. The washed samples were then stored at -65°C until further use. The moisture, protein, fat and ash contents were determined as 79.26 %, 14.41 %, 5.75 % and 0.47 % (w/w), respectively. DPPH (1,1-Diphenyl-2-picrylhydrazyl radical), salicylic acid, pyrogallol, reduced L-glutathione (GSH) and other chemicals were purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). All other chemical reagents used in this study were of analytical grade.

Experimental design

A Response Surface Methodology (RSM) and Box-Behnken design (BBD) with three variables and three levels was selected to investigate the effects of ultrasound power ($\times_1$), ultrasound treat time ($\times_2$) and extraction temperature ($\times_3$) on the UAE of porcine placenta protein. The independent variables and their levels are given in Table 1. The design assay consisted 15 experimental points including three replicates of the center point. The experimental results were fitted to the following predictive quadratic polynomial equation as the correlation between the response and the independent variables:

$$Y_i = \beta_0 + \sum \beta_i X_i + \sum \beta_{ii} X_i^2 + \sum \beta_{ij} X_i X_j \quad (1)$$

Where Y_i is the predicated response, β_0 , β_i , β_{ii} and β_{ij} are the linear, quadratic and interaction regression coefficients of variables, respectively; and X_i and X_j are independent variables.

Extraction conditions

The frozen porcine placenta was thawed and washed until no blood and impurity was observed. The sample was cut into around 0.5 cm $\times$ 0.5 cm and drained, then homogenized at 10,000 rpm for 5 min with a litter of deionized water (raw

Table 1 Experimental design of independent variables and their levels employed in Box-Behnken design for PPWP extraction

Independent variables	Symbol	Range and levels		
		−1	0	1
Ultrasound power (w)	X_1	120	240	360
Ultrasound treat time (min)	X_2	3	6	9
Extraction temperature ($^{\circ}\text{C}$)	X_3	40	50	60

material: solvent=2:3). For conventional extraction method (CEM), the homogenized material was mixed with deionized water (liquid/solid=15) and 80 mL suspension was transferred into a 100 mL jacketed beaker, and heated in a 50 °C water bath for 120 min with constant stirring. Then, the mixture was centrifuged at 8,000 g for 30 min at 4 °C and the supernatant was collected and called CEM-PPWP. For UAE method (called UAE-PPWP), the suspension was ultrasonicated at various levels of ultrasound power of 120 w, 240 w, 360 w, and ultrasound treat time 3 min, 6 min 9 min (pulse mode on 5 s, off 10 s), extraction temperature of 40 °C, 50 °C, 60 °C, as indicated in Table 1. An ultrasonic generator (JY98-IIIN Ningbo Scientz Biotechnology Co. Ltd., China) with the probe diameter of 15 mm and frequency of 20 KHz was

inserted in the center of the suspension about 2 cm deep directly.

Determination of water-soluble protein content

The water-soluble protein was determined using the Bradford assay method (Bradford 1976) with some modification. One mL sample was mixed with 5 mL protein reagent which contained 0.01 % (w/v) Coomassie Brilliant Blue G-250, 4.7 % (w/v) ethanol, and 8.5 % (w/v) phosphoric acid by vortexing, and keep at room temperature for 5 min. The mixture solution was measured at 595 nm using bovine serum albumin (BSA) as the standard. The extraction yield of porcine placenta water-soluble protein (PPWP) was calculated as follow:

$$\text{Extraction yield (\%)} = \frac{\text{volume of supernatant} \times \text{protein concentration of supernatant}}{\text{protein content of raw material}} \times 100 \quad (2)$$

Evaluation of antioxidant activity

In order to evaluate the antioxidant activity of PPWP, a number of widely used antioxidant assay methods were employed. As L-glutathione reduced (GSH) is an amino acid and also a good antioxidant (Pownall et al. 2011), it was used as a reference in this work.

DPPH radical scavenging activity assay

The DPPH radical scavenging activity of PPWP was evaluated according to the descriptions of Qin et al. (2011) and Teng et al. (2011) with slight modification. Briefly, 2 mL of PPWP was mixed thoroughly with 2 mL of 0.1 mM DPPH in ethanol, and kept in dark at room temperature for 30 min. After centrifuged at 5,000×g for 10 min, the absorbance of supernatant was measured at 517 nm. The DPPH radical scavenging capacity was determined by equation (3) given below.

$$\text{Scavenging Ability(\%)} = \left(1 - \frac{A_1 - A_2}{A_0}\right) \times 100 \quad (3)$$

where A_1 is the absorbance of 2 mL of sample+2 mL of 0.1 mM DPPH in ethanol; A_2 is the absorbance of 2 mL of sample+2 mL of ethanol, A_0 is the absorbance of 2 mL of 0.1 mM DPPH in ethanol +2 mL of distilled water.

Superoxide radical scavenging activity assay

Superoxide radicals ($O_2^{\cdot-}$) scavenging activity of PPWP was determined according to Udenigwe et al. (2009) and Zhang

et al. (2012) with slight modification. The reaction mixture containing 1 mL sample, 1 mL EDTA (1 mM), 2 mL Tris–HCl buffer (pH 8.2) was kept at ambient temperature for 10 min. Then 0.3 mL pyrogallol solution (3 mM, prepared by 10 mM HCl) was added to the reaction mixture and immediately measured at 320 nm at every 1 min for 4 min. The slope of the line of absorbance versus time was calculated for each sample and the control. The superoxide radicals scavenging activity was calculated using equation (4) given below.

$$\text{Scavenging activity(\%)} = \frac{S_c - S_s}{S_c} \times 100 \quad (4)$$

Where, S_c is the slope of the line representing absorbance versus time in the case of 1 mL distilled water instead of sample. S_s is the slope of absorbance versus time of the sample.

Hydroxyl radical scavenging activity

Hydroxyl radicals ($\cdot OH$) were generated by the Fenton reaction in a mixture containing 1 mL sample, 0.3 mL $FeSO_4$ (8 mM), 1 mL salicylic acid (3 mM), 0.25 mL H_2O_2 (20 mM) and allowed the reaction to occur at 37 °C for 1 h (Zhang et al. 2012). The reaction mixture was then cooled down to the ambient temperature. Three mL of the mixture was mixed with 0.45 mL distilled water and centrifuged at 5,000 g for 10 min. The absorbance of the supernatant was measured at 510 nm. The hydroxyl radicals scavenging activity was calculated using equation (5) given below.

$$\text{Scavenging activity(\%)} = \frac{A_c - A_s}{A_c} \times 100 \quad (5)$$

where A_c is the absorbance of supernatant using 1 mL distilled water instead of 1 mL PPWP sample in the reaction and A_s is the absorbance of supernatant using 1 mL PPWP sample.

Statistical analysis

All measurements were carried out in triplicate and analysis of variance (ANOVA) was performed to evaluate significant differences between independent variables. Difference between the mean values was considered significant at $p < 0.05$ (95 % confidence level). The statistical calculations were performed using SPSS software, version 14 (SPSS Inc., Chicago, USA). To visualize the relationships between the responses and the independent variables and also to deduce the optimum extraction conditions for maximum value of the response, the fitted quadratic polynomial equation was expressed as both response surface and contour plots.

Result and discussion

Fitting the models

Response surface methodology (RSM) is a collection of mathematical and statistical techniques that establishes a model and analysis of problems in which one or more responses of interest is influenced by several variables and optimize the conditions for a certain goal (Rafieian et al. 2013). The experimental conditions and extraction yields from porcine placenta using three factor-three level BBD are given in Table 2.

A second order polynomial equation was applied to establish a mathematical model to optimize the extraction conditions of PPWP and investigate the relationships between the process variables and the response. The equation used for the UAE of PPWP was as follows:

$$Y\% = 32.57 + 0.88X_1 + 1.56X_2 - 0.89X_3 - 1.07X_1X_2 + 0.68X_1X_3 - 0.15X_2X_3 - 1.96X_1^2 - 3.23X_2^2 - 2.98X_3^2 \quad (6)$$

The analysis of variance (ANOVA) for the quadratic polynomial model of extraction yield of PPWP is shown in Table 3. The high F-value (14.46) and low p-value (0.0045) of the model indicated that most of the variation in the response can be explained by the regression equation. The lower value of coefficient of variation (CV) was

3.31 %, suggested a good precision and reliability of the experiments. Besides, the F-value and p-value of the lack of fit were 7.72 and 0.1169, respectively, which suggested that the developed model was adequate for predicting the yield of PPWP under any combination of values of the variables. The high R^2 (0.9630) value demonstrated that the model is suitable to explain the relationship between the response and independent variables.

Apart from determination coefficient (R^2), the adequacy of the model was evaluated through various diagnostic plots such as predicted values versus actual values, normal % probability and internally studentized residuals. The experimental values were close to the predicted values on the developed model to form an almost straight line which suggested the predicted data were accord with the real data (Fig. 1a). The normal % probability plot of residuals for responses was normally distributed and approximated along a straight line (Fig. 1b). As Fig. 1c shows, all the data points lay within the limits (± 3) and the residuals scatter randomly on the display in internally studentized residuals plot. The results of all the plots (Fig. 1a, b, c) indicated that the developed model is adequate to describe the PPWP extraction yield (Prakash et al. 2013; Samavati 2013).

Analysis of the mathematical model and optimization of the process

The coefficients of the linear ($\times_1$; $\times_2$; $\times_3$), quadratic (I_1^2 ; I_2^2 ; $\times_3^2$), and interaction ($\times_1\epsilon_2$; $\epsilon_1\times_3$; $\epsilon_2\epsilon_3$) of the model equation were calculated (Table 3). The lower P-value and larger

Table 2 Box-Behnken experimental design matrix with observed and predicted values

Run order	Ultrasound power (w)	Ultrasound treat time (min)	Extraction temperature (°C)	PPWP extraction yield (%)	
				Experimental	Predicted
1	−1	−1	0	23.6	23.9
2	−1	1	0	30.1	29.1
3	1	−1	0	26.8	27.8
4	1	1	0	29	28.7
5	0	−1	−1	26.2	25.5
6	0	−1	1	24.6	24.0
7	0	1	−1	28.4	28.9
8	0	1	1	26.2	26.9
9	−1	0	−1	27.9	28.3
10	1	0	−1	29	28.7
11	−1	0	1	24.9	25.2
12	1	0	1	28.7	28.3
13	0	0	0	32.7	32.6
14	0	0	0	32.1	32.6
15	0	0	0	32.9	32.6

Table 3 Analysis of variance for regression model of PPWP extraction yield

Model term	DF	Sum of squares	Mean square	F VALUE	P-value
x_1	1	6.13	6.13	7.03	0.0454
x_2	1	19.53	19.53	22.40	0.0052
x_3	1	6.30	6.30	7.23	0.0434
$x_1 x_2$	1	4.62	4.62	5.30	0.0695
$x_1 x_3$	1	1.82	1.82	2.09	0.2079
$x_2 x_3$	1	0.090	0.090	0.10	0.7610
x_1^2	1	14.16	14.16	16.24	0.0100
x_2^2	1	38.60	38.60	44.28	0.0012
x_3^2	1	32.86	32.86	38.69	0.0017
Model	9	113.43	12.60	14.46	0.0045
Lack of fit	3	4.01	1.34	7.72	0.1169
Error	2	0.35	0.17	-	-
Total	14	117.79	-	-	-
R-square	0.9630	R _{Adj} -square	-	0.8964	
C.V%	3.31	-	-	-	-

F-value means the corresponding variables are more significant (Amin and Anggoro 2004). Therefore, ultrasound treat time (x_2) had the most significant ($P < 0.01$) and ultrasound power (x_1) and extraction temperature (x_3) had significant effects on the extraction yield ($P < 0.05$). The quadratic terms of x_1^2 , x_2^2 and x_3^2 revealed a highly significant effect on extraction yield ($P \leq 0.01$). However, all the interaction terms ($x_1 x_2$, $x_1 x_3$, $x_2 x_3$) were not found to contribute to the response at a significant level ($P > 0.05$).

The objective of optimization work was to gain the conditions at maximum extraction yield of PPWP. Precise coordinates of maximum were obtained by calculating the partial derivatives of regression Eq. (6), which were set to zero, and three equations would form:

$$3.92X_1 + 1.07X_2 - 0.68X_3 = 0.88 \quad (7)$$

$$1.07X_1 + 6.46X_2 + 0.15X_3 = 1.56 \quad (8)$$

$$0.68X_1 - 0.15X_2 - 5.96X_3 = 0.89 \quad (9)$$

Using equations (7)–(9) the following results were obtained: $x_1 = 0.138$, $x_2 = 0.222$, $x_3 = -0.139$, which means the optimal UAE conditions: ultrasound power, 257 w; ultrasound treat time, 7 min; extraction temperature, 49 °C. Under those conditions the extraction yield of PPWP was 32.9 %, and a desirability value of 0.996.

Validation of the model

For validation of the model, the PPWP was extracted under optimal UAE conditions, and compared between the

experimental and predicted values (Table 4). Calculated from the model, the maximum extraction yield of PPWP was 32.9 %, while the experimental extraction yield under the same conditions was 32.7 ± 0.4 %. These results demonstrated that the established model could be used to predict the extraction yield of PPWP using the UAE method, and validation of the optimized conditions.

Effects of process variables on response

As Fig. 2a, c shows, the extraction yield of PPWP was significantly increased by extending ultrasound treat time (x_2) within 7 min ($P < 0.01$), which means with the time increase, more porcine placenta cells were broken and water-soluble proteins were released due to the acoustic cavitation of ultrasound. Nevertheless, there was no further increase of the extraction yield with excessive extension of the time, which could attributed to the releasing of water-soluble proteins reached to the maximum UAE efficiency, and this phenomenon was also observed by Zhu et al. (2009) when extraction of defatted wheat germ proteins using ultrasound-assisted method.

With the increase of ultrasound power (x_1), the extraction yield of PPWP also increased (Fig. 2b). This could attribute to the increased ultrasound power made a more intense mass transfer in the liquid/solid system. However, when the ultrasound power was above 257w, the extraction yield did not increase further, which suggested that an equilibrium state may have been reached in the extraction system. This observation was in agreement with Karki et al. (2010) in high-power ultrasound extraction of protein and sugar from defatted soy flakes.

Fig. 1 Diagnostic plots for the model adequacy, **(a)** The plot of predicted versus actual extraction yield of PPWP. **(b)** The plot of normal % probability. **(c)** The plot of internally studentized

Table 4 Optimum UAE conditions for extraction of PPWP, predicted and experimental values of extraction yield of

Optimum UAE parameters			The UAE extraction yield (%)	
Ultrasound power (w)	Ultrasound treat time (min)	Extraction temperature (°C)	Predicted	Experimental
257	7	49	32.9	32.7±0.4

When temperature was lower than 49 °C, with the increase of extraction temperature, the extraction yield of PPWP increased. While the extraction temperature was above 49 °C, the extraction yield dropped rapidly. Ma et al. (2010) investigated the effect of temperature on the solubility of peanut protein concentrates in which the increase of temperature above a certain limit resulted in the decrease of protein solubility dramatically, which suggested that proteins are sensitive to high temperatures and

a suitable temperature should be selected in extraction of proteins.

The interaction effects of ultrasound power (x_1) and ultrasound treat time (x_2) on the extraction yield of PPWP are shown in Fig. 2a, while experimental extraction temperature was fixed on middle level of 50 °C. As the response surface plot and their corresponding counter plot (Fig. 2a) shows, there is no significant effect of ultrasound power on the extraction yield of PPWP at low level of ultrasound treat time.

Fig. 2 Response surface and contour plots for the effects of ultrasound power (x_1), ultrasound treat time (x_2), and extraction temperature (x_3)

Fig. 2 (continued)

Furthermore, at the middle level of ultrasound treat time and ultrasound power, the maximum extraction yield of PPWP (32.9 %) was obtained, while the increased of ultrasound treat time and ultrasound power, there was no increase of extraction yield.

Fig. 2b presents the interaction effect of ultrasound power ($\times_1$) and extraction temperature ($\times_3$) on the extraction yield of PPWP under settled experimental ultrasound treat time at its middle level (6 min). It was obvious that the extraction of PPWP increased with ultrasound power when the extraction temperature at middle level and the maximum extraction yield of PPWP was reached in the range of ultrasound power 240–270 W and extraction temperature 47.5–50 °C.

As we can see in Fig. 2c, with the increase of extraction temperature, the extraction yield of PPWP did not significantly increase at the low level of ultrasound treat time. With the increase in ultrasound treat time, the

extraction yield of PPWP increased from 28 % to 32 % at the middle level of extraction temperature, while increase only from 30 % to 32 % at the middle of level of ultrasound treat time with the increase of extraction temperature. This indicated that the increase of both the ultrasound treat time and the extraction temperature could improve the UAE efficiency of PPWP, however, the effect of ultrasound treat time was greater than extraction temperature on the extraction yield of PPWP.

Comparative study of the PPWP properties extracted from conventional method and UAE

The extraction efficiency of PPWP is closely related to their extraction conditions. The extraction yield and antioxidant activity of PPWP using CEM and UAE

Fig. 2 (continued)

(Fig. 3 and Fig. 4) were compared. In terms of extraction yield, there was a remarkable increase by the UAE method, which might be due to the ultrasound intensified both the mass transfer in the liquid/solid system and intraparticle diffusion, while CEM stirring only intensify the external mass transfer (Esclapez et al. 2011). Besides, high intensity ultrasound increased protein solubility by breaking the small aggregates in extraction solution so that increasing protein-water interactions (Arzeni et al. 2012a), hence, increase the water-soluble proteins of porcine placenta. At optimal conditions of UAE and CEM (the data of optimization process not shown), the extraction yield of UAE was twice to that of CEM and the extraction time of CEM was about 17-fold than that of UAE. The more effective

UAE of bioactives have also been reported by a number of investigators (Bagherian et al. 2011; Karki et al. 2010; Picó 2013).

Antioxidant activity of porcine placenta extracts

The effectiveness of antioxidants depends on many factors and the antioxidant activity of a compound can be better characterized by using different assays based on different mechanisms (Xie et al. 2008). In the present study, the antioxidant activity of porcine placenta extract was evaluated by its scavenging effects of DPPH (1,1-diphenyl-2-picrylhydrazyl) radical, superoxide anion radical and hydroxyl radical.

DPPH is oil-soluble that has been used extensively as a free radical to evaluate the reducing substances (Wang et al. 2012),

Fig. 3 Comparison UAE with CEM on the extraction yield and extraction time

while the superoxide and hydroxyl radicals are water-soluble radical species and also been used widely to measure the antioxidant capacity of antioxidants (Luo et al. 2013; Qin et al. 2011). As shows in Fig. 4, the higher the concentrations of PPWP, the higher level of free radicals scavenging activities were observed. The DPPH radical scavenging of PPWP reached above 70 % at 3 mg/mL which was similar to that of the GSH at 0.2 mg/mL. Also, the scavenging activity of superoxide and hydroxyl radicals of PPWP had a similar trend to that of DPPH, and the scavenging value of those two radicals at the concentration of 5 mg/mL were 60 % and 55 %, respectively, which was also close to the scavenging activity of GSH at 0.2 mg/mL.

On the other hand, the antioxidant capacity of PPWP from CEM and UAE methods had not changed significantly (Fig. 4), which may due to the sulfhydryl content of the protein, such as free -SH group and -S-S bond (Žilić et al. 2012), was unchanged during the ultrasound treatment. The sulfhydryl content is closely correlated with protein structure and conformation (Arzeni et al. 2012a) and even has a protective effect when sample under ultrasound treatment so make them less susceptible to degradation (Hu et al. 2013). Besides, the scavenging DPPH radical activity of PPWP from UAE was higher than that from CEM at any concentration (Fig. 4), which may attribute to the fact that ultrasound treatment lead to an increase of surface hydrophobicity of the PPWP (Arzeni et al. 2012a; Arzeni et al. 2012b) that facilitate more antioxidants (PPWP) accessible by DPPH radicals (Zhu et al. 2008). The scavenging superoxide and hydroxyl radical activity of PPWP from UAE were higher than that from CME might also due to this reason.

Fig. 4 Compared the scavenging ability of GSH, UAE-PPWP, CEM-PPWP on the DPPH (a), $\cdot\text{O}_2$ (b) and OH c

Conclusions

In this work, UAE was optimized for the extraction of porcine placenta water-soluble protein. Three factors at three levels of Box-Behnken response surface experimental design was successfully employed to optimized the individual and interactive effects of process variables on the maximum extraction of water-soluble proteins from porcine placenta. The results suggested that UAE could not only reduce the extraction time, but also increase the extraction yield from 17.7 % to 32.7 %, compared with the conventional extraction method. Meanwhile, the antioxidant activity of extracts from UAE were higher than those from conventional method, although not significantly, indicated that UAE had a positive effect or at least no negative effect on the bioactivity of porcine placenta water-soluble protein.

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