Antioxidant and functional properties of tea protein as affected by the different tea processing methods

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Abstract The Box-Behnken design combined with response surface methodology was used to optimize alkali extraction of protein from tea. Three independent extraction variables (extraction time: X_1 ; extraction temperature: X_2 ; alkali concentration: X_3) were evaluated. The antioxidant and functional properties of tea protein as affected by different tea processing were compared. The optimum conditions were: extraction time of 85 min, extraction temperature of 80 °C, and alkali concentration of 0.15 M. Under such conditions, the predicted maximum protein extracted yield was 20.73 %, which coincided with the experimental values. Three kinds of tea protein showed good antioxidant and functional properties. Tea processing method had a significant impact on the properties of tea protein. Protein from oolong tea showed the best properties and it might be used as a good source of potential antioxidant and additives in food industry.

Keywords Tea protein · Extraction · Response surface methodology · Antioxidant · Functional properties

Introduction

Tea (*Camellia sinensis*) is the second most popular drink next to water, which was initially cultivated in China and India. There are several kinds of tea according to the fermentation process. Green tea production was obtained from freshly harvested tea leaves with inactive enzymatic oxidation, while the production of oolong and black tea was made by controlling the oxidation by the leaf enzymes during the fermentation (Balentine et al. 1997). The nutritional and medicinal values of tea were highly praised because of its bioactivities, such as

metabolism accommodation activities, antioxidant activities, anticancer activities and protective effect in cardiovascular disease (Christiane and Edward 2001). There are many bioactive constituents including polyphenols, flavones, polysaccharides, vitamins, and some amino acids existed in tea (Christiane and Edward 2001). The previous studies mainly focused on the polyphenols and tea catechins have been reported to possess various physiological and pharmacological properties, including effective antioxidants that scavenge free radicals, antifungal activities, antibacterial activities and anticancer activities (Tsai et al. 2013). It was shown that tea contained around 21–28 % of protein on dry-base (Gu et al. 2002), but tea protein was seldom investigated till in recent years. It was recently reported that tea protein has bioactive properties, such as antioxidant (Wang and Hu 2005), anti-mutation (Bu-Abbas et al. 1994), protecting biological cells against mutagenesis caused by irradiation (Li et al. 2001). Tea is mainly consumed in three forms including green tea, oolong tea and black tea according to the different fermentation process. However, there were no comparative studies on the functional properties or the bioactivities of the proteins from the three kinds of teas.

Proteins are widely applied in food and pharmaceutical industry due to the polarized distribution of hydrophobic and hydrophilic groups along the back bone. Proteins also have special uses as foaming agents or as functional ingredients for nutrient delivery in foods (Shen et al. 2008). So many researchers are focused on the studies of new proteins with good functional properties. The functional properties and applications of protein are affected by their isolation and concentration methods. Most protein were only soluble in high pH solution because of their hydrophobic nature and the disulphide bonding between protein molecules, which could be broken down in high alkali concentration, so that alkali method has been widely used in protein extraction from plant sources. It was further studied that its effectiveness could be influenced of the extraction conditions such as extraction time, extraction temperature, alkali concentration and solvent/material ratio (Sun

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and Tian 2003). Shen et al. (2008) extracted proteins from tea leave pulps using alkaline and enzyme method. In addition, alkaline method was also applied in the extractions of many kinds of protein samples from different sources, such as red pepper seed, pigeon pea and peanut (Firatligil-Durmus and Evranuz 2010; Mizubuti et al. 2000; Wang et al. 2009). Response surface methodology (RSM) is an effective tool for optimizing the process when many factors and interactions affect the desired response (Fang et al. 2012). However, there are no reports on the isolation of tea protein by using RSM till now.

The purpose of the present work was to optimize the process for the extraction of protein from tea employing a three-level, three-variable central composite rotatable design. The antioxidant activities and functional properties of the proteins extracted under the optimum conditions from three different kinds of tea (green tea, oolong tea and black tea) were also comparatively studied.

Materials and methods

Materials

Green tea, oolong tea, and black tea were obtained from Huangshan Mountain Tea Factory, (Anhui, China) and they were processed with the same freshly harvested tea leaves. All the three kinds of tea belong to coarse tea. 1, 1-Diphenyl-2-picrylhydrazyl (DPPH) was obtained from Sigma-Aldrich (Shanghai, China). All other chemicals and reagents were purchased locally and were of analytical grade.

Experimental design

On the basis of the single factor experiment, the solvent/material ratio and the independent variable of each of the extraction were determined, respectively. Box-Behnken design (BBD) with three independent variables was used for the optimization. Protein was extracted from tea with selected 15 combinations of independent variables such as extraction time (X_1 : 60 min (−1), 120 min (0) and 180 min (+1)), extraction temperature (X_2 : 40 °C (−1), 65 °C (0) and 90 °C (+1)) and alkali concentration (X_3 : 0.05 M (−1), 0.1 M (0) and 0.15 M (+1)), meanwhile solvent/material ratio was controlled of 40:1 (v/m). Fifteen experiments were augmented with three times and carried out at the center points to evaluate the pure error.

A second-order polynomial regression model was used to express the yield as a function of the independent variables as follows:

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

where Y is the response function, where β_0 is defined as the constant, β_i the linear coefficient, β_{ii} the quadratic coefficient and β_{ij} the cross product coefficient. And accordingly X_i and X_j represent the coded independent variables. The variables were coded according to the equation:

$$x_i = \frac{X_i - X_0}{\Delta X}, \quad (2)$$

where x_i is the (dimensionless) coded value of the variable X_i , X_0 is the value of X_i at the center point, and ΔX is the step change (Wang et al. 2007).

Protein extraction

Protein samples were extracted from green tea, oolong tea and black tea (15 g/time × 3 times) under the optimum extraction conditions. The three kinds of proteins were then purified by isoelectric point (pI) precipitating method, washed by ethanol, dia and then lyophilized, which were named as GTP, OTP and BTP, respectively. The three protein samples were then comparatively analyzed in the following studies.

Compositional analysis

The procedure used in determining the total protein content was adapted from the Bradford method (Bradford 1976). One hundred μ L of sample solution (1 mg/mL) was mixed with 5 mL of Bradford Protein Assay kit solution. After shaking and incubating in room temperature, the absorbance was measured at 595 nm, and the protein content was calculated with bovine serum albumin (BSA) as standard protein. Phenol–sulfuric acid method (Dubois et al. 1956) was employed for the measurement of total sugar content with glucose as the standard. Uronic acid content was determined by carbazole-sulfuric acid method (Bitter and Muir 1962) with D-galactose as standard. Total phenolics content was detected using gallic acid as standard according to our previous study (Chen et al. 2012).

Molecular weight analysis by SDS-PAGE

The protein samples were analyzed by SDS-PAGE (Laemmli 1970) with 10 % separating gel and 5 % stacking gel, while mercaptoethanol was added to eliminate the affect of advanced structure on molecule weight. Protein samples and molecular weight markers were loaded onto SDS gel containing 5 % acrylamide in stacking gel and 10 % acrylamide in separating gel. Electrophoresis was carried out with running buffer (25 mM Tris, 190 mM glycine, 10 % SDS) at 100 V for stacking gel and 200 V for separating gel. Bands were stained using Coomassie brilliant blue.

Antioxidant activities assay

Scavenging activity against DPPH free radicals

Scavenging activity of tea protein on DPPH free radicals was according to our previous studies (Fu et al. 2010). Briefly, 100 μ L sample aqueous solutions were mixed with 2.9 mL of 120 μ M DPPH solution, and then the mixture was incubated for 30 min at 37 °C. The absorbance of the mixture was measured at 517 nm. The scavenging activity was explained by the following equation:

$$I\% = \frac{A_{\text{blank}} - A_{\text{sample}}}{A_{\text{blank}}} \times 100\% \quad (3)$$

Ferric reducing power (FRP)

The FRP potential was determined according to the method of Zhou et al. (2012). One hundred microliter sample aqueous solutions were mixed with 0.7 mL of PBS (0.2 M, pH 6.6) and 2 mL of aqueous potassium hexacyanoferrate ($K_3[Fe(CN)_6]$) solution (30 mM). After incubating for 20 min at 50 °C, 2 mL of 10 % trichloroacetic acid was added into the mixture and kept for 10 min. Then 1 mL of reaction solution mixed with 3 mL of 1.7 mM $FeCl_3$ and the absorbance of the mixture was measured at 700 nm.

Effects of tea protein on lipid peroxidation

The inhibition of lipid peroxidation was assayed according to our previous studies (Chen et al. 2008). Kunming mice weighing 20 ± 2 g (purchased from Beijing Weitonglihua Company, Beijing, China) were anesthetized using diethyl ether and the abdomen was opened and the liver was quickly removed. The livers were then cut into small pieces and homogenized in phosphate buffer (50 mM, pH 7.4) with a homogenizer to give a 10 % (w/v) liver homogenate. The liver homogenate was further centrifuged at $3000 \times g$ for 10 min. The supernatant of the liver homogenate was collected and the amount of protein was determined by the method of Bradford (1976). The extent of lipid peroxidation

was evaluated by measuring the product of thiobarbituric acid reactive substances (TBARS) in the rat liver homogenate. The reaction mixture was composed of 0.5 mL of tissue homogenate, 0.9 mL of phosphate buffer (50 mM, pH 7.4), 0.25 mL of $FeSO_4$ (0.01 mM), 0.25 mL of ascorbic acid (0.1 mM), and 0.1 mL of different concentration of the protein samples and the standard sample. The reaction mixture was incubated at 37 °C for 30 min and the reaction was then terminated by adding 1 mL of TCA (20 % w/v) to the mixture. After centrifugation at $3000 \times g$ for 15 min, the supernatant was incubated with 1 mL of TBA (0.67 %) at 100 °C for 15 min. The absorbance of the complex was measured at 532 nm.

Functional properties assay

Solubility assay

The solubility of samples was evaluated according to Guan et al. (2007) with some modification. Twenty milligrams samples were suspended in 2 mL distilled water and the pH of the system was adjusted to the desired values (5.0, 7.0, 9.0, 11.0 and 13.0) using either 0.1 M HCl or 0.1 M NaOH. The suspension was stirred 30 min at room temperature, and then centrifuged at $3000 \times g$ for 30 min. The Bradford method was used to determine the protein contents of the supernatants.

$$\text{Solubility} = \frac{\text{Weight of protein in the supernatants (g)}}{\text{Weight of protein in sample (g)}} \times 100\% \quad (4)$$

Water absorption capacity (WAC)

The water absorption capacity of samples was evaluated according to Guan et al. (2007) with little modification. Briefly, 50 mg of samples were dispersed in 5 mL distilled water with a vortex mixer for 30 s in graduated centrifuge tubes. After standing for 6 h at room temperature, the dispersions were centrifuged at $3000 \times g$ for 15 min. The volume of the supernatants was determined, and WAC was expressed as mL of water absorbed per g of protein sample.

$$WAC = \frac{\text{Volume of added deionized water} - \text{Volume of supernatant (mL)}}{\text{Weight of protein sample (g)}} \times 100\% \quad (5)$$

Oil absorption capacity (OAC)

The oil absorption capacity of samples was evaluated according to Guan et al. (2007) with little modification.

Briefly, 50 mg of samples were dispersed in 3 mL oil with a vortex mixer for 30 s in graduated centrifuge tubes. After standing for 6 h at room temperature, the dispersions were centrifuged at $3000 \times g$ for 15 min. The volume of the

supernatants was determined, and OAC was expressed as mL of oil absorbed per g of protein sample.

$$\text{OAC} = \frac{\text{Volume of added oil} - \text{Volume of supernatant (mL)}}{\text{Weight of protein sample (g)}} \times 100\% \quad (6)$$

Emulsifying capacity (EC) and emulsifying stability (ES) assay

Emulsifying properties were determined according to the methods of Koli et al. (2012). Two microlitres of protein solution (20 mg/mL) was homogenized at medium speed for 60 s, and then added 2 mL of soybean oil homogeneous 60 s (The homogenizer equipped with a motorized stirrer driven by a rheostat Ultra-T18 homogenizer, Shanghai, China). The emulsion was shifted into a centrifuge tube with scale, centrifuged 2700×g for 5 min, and then the volume of emulsion layer was read out.

$$\text{EC} = \frac{\text{Volume of emulsion layer (mL)}}{\text{Volume of liquid in the tube (mL)}} \times 100\% \quad (7)$$

Then the emulsion was heated at 80 °C for 30 min and chilled to room temperature, centrifuged 2700×g for 5 min, read out the volume of remainder emulsion layer.

$$\text{ES} = \frac{\text{Volume of remainder emulsion layer (mL)}}{\text{Volume of initial emulsion layer (mL)}} \times 100\% \quad (8)$$

Foaming capacity (FC) assay

Foaming capacity was determined according to the methods of El-Beltagy and El-Sayed (2012).

Two microlitres of protein solution (20 mg/mL) was homogenized for 30 s, and then the volume of ultimate liquid was measured.

$$\text{FC} = \frac{\text{Volume of liquid increased (mL)}}{\text{Volume of ultimate liquid (mL)}} \times 100\% \quad (9)$$

Gelating capacity (GC) assay

Gelating capacity was determined according to the methods of Wu et al. (2008). Two microlitres of protein solution (4, 8, 12, 16, 20, 40, 60, 80, and 100 mg/mL) was heated in 100 °C for 1 h, rapidly cold at 4 °C for 2 h. The minimum concentration would be the minimum gelling concentration when the tube was inverted without the liquid falling out.

Statistical analysis

All measurements were run in triplicate, and data were expressed as means ± standard deviation (SD). The difference was evaluated using Students't-test. P value less than 0.05 was considered statistically significant.

Results and discussion

Optimization of extraction conditions

Statistical analysis and the model fitting

Response surface optimization was widely used in the extraction of natural products, which can establish a high precision regression equation and detail the interactions between several factors (Martínez and Pilosof 2012). Green tea was extracted for its protein following 15 combinations of three independent variables (extraction time, temperature and alkali concentration) (Table 1). The analytical values of the independent process variables (X_1 , X_2 , and X_3) considered, as well as the experimental and predicted values for response (extraction yields of protein) are given in Table 2. Results revealed that the experimental pure protein yield varied from 3.14 to 19.73 g soluble protein/100 g green tea.

The application of RSM yields the following regression equation, which is an empirical relationship between protein yield and the test variable in coded units, as given in the following equation.

$$Y = 12.71 + 0.95375X_1 + 3.39125X_2 + 3.8X_3 - 0.87875X_1^2 - 0.4075X_1X_2 - 3.30875X_2^2 + 0.71X_2X_3 + 0.54875X_3^2 \quad (10)$$

where Y is the yield of protein and X_1 , X_2 , and X_3 are the values for extraction time, extraction temperature, and alkali concentration, respectively.

The statistical significance of the regression model was checked by F-test and p-value, and the analysis of variance (ANOVA) for the response surface quadratic model was shown in Table 3. The total determination coefficient (R^2) was 92.72 %, thus, the model was able to explain more than 80 % of the observed response showed by ANOVA of the quadratic regression model, indicating that the model was adequate for prediction within the range of experimental variables (Martínez and Pilosof 2012).

The results suggested that the model, as evident from the calculated F value (265.0987) and the low probability value ($P < 0.05$), are adequate for predicting within the range of the variables employed.

Table 1 Central composite design arrangement, responses and predicted values for protein yield

RUN	Coded variables			Uncoded variables			Protein yield (Y), %	
	X ₁	X ₂	X ₃	X ₁	X ₂	X ₃	Experimental	Predicted
1	−1	−1	0	1	40	0.1	4.56	3.77
2	−1	1	0	1	90	0.1	12.30	11.37
3	1	−1	0	3	40	0.1	5.56	6.49
4	1	1	0	3	90	0.1	11.67	12.46
5	0	−1	−1	2	40	0.05	4.87	3.47
6	0	−1	1	2	40	0.15	8.39	9.65
7	0	1	−1	2	90	0.05	10.09	8.83
8	0	1	1	2	90	0.15	16.45	17.85
9	−1	0	−1	1	65	0.05	6.31	7.63
10	1	0	−1	3	65	0.05	8.19	9.53
11	−1	0	1	1	65	0.15	14.82	15.23
12	1	0	1	3	65	0.15	22.20	17.13
13	0	0	0	2	65	0.1	12.07	12.71
14	0	0	0	2	65	0.1	13.22	12.71
15	0	0	0	2	65	0.1	12.84	12.71

The P-values were used as a tool to check the significance of each coefficient, and the smaller the p-value was, the more significant the corresponding coefficient was (Guo et al. 2010). Extraction temperature and alkali concentration were the most significant factors ($p < 0.01$), while time was not significant factor (Table 3). The results didn't accord with the reports of Shen et al. (2008), in which the extraction time was the most important factor in the orthogonal experiment. This might due to the different method and different kind of tea were used. This phenomena was also found in other studies, such as pH value was the significant factor for red pepper protein extraction (Firatligil-Durmus and Evranuz 2010) while extraction temperature was the significant factor for the extraction of peanut protein (Rustom et al. 1991). In addition, the quadratic term coefficients (X_2^2) were also found significant ($p < 0.05$), illustrated the significant influence of

temperature again. The other term coefficients (X_1 , X_1^2 , X_3^2 , X_1X_2 , X_1X_3 , X_2X_3) were not significant ($p > 0.05$).

Optimization of extraction conditions

Response surfaces were plotted using SAS version 8.0 software and were used to study the effects of parameters and their interactions on the yield of tea protein. The results of extraction yield of protein affected by extraction temperature, time and alkali concentration are presented in Fig. 1. These types of plots showed the effects of two factors on the response at a time and the other factor was kept at level zero. The maximum value predicted by the surface was confined in the smallest ellipse in the contour diagram. Elliptical contours were obtained when there was a perfect interaction between the independent variables.

Table 2 Estimated regression model of relationship between response variables (Y) and independent variables (X₁, X₂, X₃)

Source	Degrees of freedom	Sum of squares	Mean square	F-value	P-value
X ₁	1	7.277112	7.277112	1.748262	0.243349
X ₂	1	92.00461	92.00461	22.10329	0.00533
X ₃	1	115.52	115.52	27.75265	0.003277
X ₁ *X ₁	1	2.851206	2.851206	0.684977	0.445569
X ₁ *X ₂	1	0.664225	0.664225	0.159574	0.706041
X ₁ *X ₃	1	3.0625	3.0625	0.735738	0.430217
X ₂ *X ₂	1	40.42274	40.42274	9.711205	0.026363
X ₂ *X ₃	1	2.0164	2.0164	0.484422	0.517444
X ₃ *X ₃	1	1.111852	1.111852	0.267113	0.627315
Model	9	265.0987	29.45541	7.0764	0.022115

Table 3 Predicted and experimental values of the responses at optimum conditions

	Extraction time (min)	Extraction temperature (°C)	Alkali concentration(M)	Yield of protein (%)
Optimum conditions	85.14428	79.795	0.15	19.47
Modified conditions	85	80	0.15	20.73±1.74 ^a

^a Mean ± standard deviation (n=5)

The 3-D response surface plot and the contour plot in Fig. 1a and d, which give the extraction yield of protein as a function of extraction time and temperature at fixed alkali concentration (0.1 M), indicated that the extraction yield of protein increased with the increasing of extraction time from 60 to 85 min, but beyond 85 min, the extraction yield of protein changed little with the increase of the extraction time, and the extraction yield of protein was found to increase rapidly with the increase of the extraction temperature from 40 to 80 °C, then decreased rapidly from 80 to 90 °C.

Figure 1b and e show the 3-D response surface plot and the contour plot at varying extraction time and alkali concentration at fixed extraction temperature 65 °C. The tendency of the extraction yield of protein changing with alkali concentration was different from the others, the yield increased with the increasing of alkali concentration and there was no maximum value obtained.

The quadratic and linear effects of extraction temperature were embodied, meanwhile the linear effect of alkali concentration time were reflected in Fig. 1c and f. The maximum extraction yield of protein was achieved when the extraction temperature and alkali concentration were 80 °C and 0.15 M, respectively.

Among the three extraction parameters that have been studied, alkali concentration was the most significant factor that affects the yield of protein, followed by the extraction temperature and time according to the regression coefficients significance of the quadratic polynomial model (Table 3) and gradient of slope in the 3-D response surface plot (Fig. 1).

In the limits of experimental conditions, obtained using MATLAB software, the optimal values of the tested variables for obtaining a protein yield of the optimal values of the tested variables for obtaining a protein yield of 19.74 % can be predicted as follows: the extraction time of 85.14428 min, the extraction temperature of 79.795 °C, and the alkali concentration 0.15 M. However, considering the operability in actual production, the optimal conditions can be modified as follows: the extraction time of 85 min, the extraction temperature of 80 °C, and the alkali concentration of 0.15 M. The alkali concentration was a little higher than the study of Shen et al. (2008), This might be due to the different resource of tea.

To validate the adequacy of the model equations, a verification experiment is carried out under the optimal conditions mentioned above. Under those conditions, the experimental yield was 20.73±1.74 % (n=5), which was well-

Fig. 1 Contour plots and response surface (3-D) showing the effect of the extraction time, extraction temperature, and alkali concentration on the response Yield. (1) Contour plots, **a** Effect of time and temperature on protein yield with alkali concentration 0.1 M; **b** Effect of time and alkali concentration on protein yield with extraction temperature 65 °C; **c** Effect of temperature and alkali concentration on protein yield with extraction time 2 h. (2) Response surface (3-D), **d** Effect of time and temperature on protein yield with alkali concentration 0.1 M; **e** Effect of time and alkali concentration on protein yield with extraction temperature 65 °C; **f** Effect of temperature and alkali concentration on protein yield with extraction time 2 h

matched with the predicted value obtained from actual experiments (Table 4). The results demonstrated the validation of the extraction model.

Protein extraction from three kinds of tea

Under the optimum conditions, three protein samples were extracted from green tea (GTP), oolong tea (OTP) and black tea (BTP). There was no color reaction detected by the spectrophotometry methods, which suggested there was no tea polyphenols existed in the tea protein samples. As shown in Table 5, the extraction yields of protein from green tea, oolong tea and black tea were 26.19 %, 23.01 % and 41.93 %, respectively. There were significant difference among the three tea protein yields ($p < 0.05$ between OTP and GTP, $p < 0.01$ between BTP and GTP), which might due to the fermentation process of tea productions (Balentine et al. 1997). The content of protein decreased in the order of OTP (527.67 mg/g), BTP (485.25 mg/g) and GTP (461.23 mg/g). This order might suggest that the control of the oxidation by the leaf enzymes during tea processing was beneficial to the extraction of these components.

The existence of small amount of total sugar and uronic acid illustrated that isoelectric point precipitation method was not good enough to isolate protein from all the water-soluble components, and the purification of protein samples should be studied. However, it may be the most useful and handy method in the extraction of protein from tea (Zhang et al. 2005). Meanwhile, the contents of them decreased in the order of OTP, BTP and GTP. The order may suggest that the process by controlling the oxidation by the leaf enzymes was benefit for the extraction of these components. Besides, there was no coloration change of the total phenol test, which illustrated that there was no phenol existed in the three protein samples.

Molecular weight analysis by SDS-PAGE

Figure 2 showed the spread of molecular weight of GTP, OTP and BTP. As the picture shown, all of the three protein extracts had the same protein structure segment with molecular weight of approximately 66.4–64.8 kDa, which was quite different from Shen's results (2008), in which enzyme methods were used. In this study, β -mercaptoethanol and SDS were used in

the buffer of tea proteins. So the conformation of the protein was destroyed and the molecular weight sizes of the protein were determined. The results of the SDS-PAGE suggested that the primary structure of the three proteins from green tea, oolong tea and black tea were the same. The conformations of the proteins need further study.

Antioxidant activities

Scavenging activity against DPPH radicals

The scavenging rate of the tea protein samples changed with different concentrations and there were dose-dependents of GTP with the concentration ranging from 30 $\mu\text{g/mL}$ to 660 $\mu\text{g/mL}$, as well as OTP and BTP (Fig. 3a). There was significant difference between the scavenging DPPH radical ability of OTP and GTP ($p < 0.05$), whereas more obvious difference between the scavenging DPPH radical ability of BTP and GTP ($p < 0.01$). GTP, OTP and BTP showed linear correlations between concentration and scavenging DPPH radical ability with the coefficient R^2 of 0.7299, 0.7843, and 0.8847, respectively. The half inhibition concentration (IC_{50}) of three protein samples increased in the order of OTP (34.01 $\mu\text{g/mL}$), GTP (34.26 $\mu\text{g/mL}$), and BTP (168.12 $\mu\text{g/mL}$), which indicated that OTP had the highest scavenging DPPH radical capacity and BTP had the lowest one. The DPPH radical scavenging capacity of the three protein was much higher than the other reported plant protein such as rapeseed protein ($\text{IC}_{50} = 2.30\text{--}3.02 \text{ mg/mL}$) (Yoshie-Stark et al. 2008). It was suggested that tea protein was a good plant antioxidant resource. DPPH, a stable radical was used to evaluate the antioxidant activity of protein samples. The mechanism is that if there was an antioxidant substance reacted with DPPH free radicals, the absorbance at 517 nm decreased, as the solution color would turn light yellow from purple (Ma et al. 2012). The antioxidant mechanism might be tea protein acting as electron donors, react with DPPH free radicals to convert them to more stable products and terminate radical chain reactions.

Ferric reducing power (FRP)

Measurement of ferric reducing power is a model usually used to study the antioxidant property of natural product extracts

Table 4 Yields and the contents of water-soluble components of protein extracted from 3 kinds of tea

Sample	Yield ^a (%)	Contents ^a (mg/g)		
		Protein	Uronic acid	Total sugar
GTP	26.19 $\pm$ 0.55	461.23 $\pm$ 6.20	144.47 $\pm$ 4.86	104.47 $\pm$ 3.25
OTP	23.01 $\pm$ 1.02 ^b	527.67 $\pm$ 8.05 ^c	173.05 $\pm$ 2.85 ^c	146.82 $\pm$ 3.69 ^c
BTP	41.93 $\pm$ 1.25 ^c	485.25 $\pm$ 9.02 ^b	153.10 $\pm$ 2.23	128.02 $\pm$ 5.23 ^b

^a Mean $\pm$ standard deviation ($n=3$)

^b $p < 0.05$ vs. Green Tea

^c $p < 0.01$ vs. Green Tea

Table 5 Some functional properties of protein extracted from three kind of tea

	GTP	OTP	BTP
WAC ^a /mL·g ⁻¹	1.26±0.07	1.36±0.07	1.33±0.034 ^b
OAC ^a /mL·g ⁻¹	1.54±0.10	1.35±0.07	1.58±0.17
Emulsifying index ^a /%	12.08±0.59	7.67±0.24 ^c	8.33±0.85 ^c
Emulsifying stability ^a /%	19.26±1.05	41.88±1.88 ^c	25.67±2.29 ^c

^a Mean ± standard deviation (n=3)^b *p*<0.05 vs. Green Tea^c *p*<0.01 vs. Green Tea

(Sivapriya and Srinivas 2007; Zhang et al. 2011). Seen from Fig. 3b, all of the three protein samples showed good FRP potentials. Reducing capacity ($A_{700\text{ nm}}$) of tea proteins increased with increase in concentration (15–180 μM). The results were in accordance with the study of Srivastava et al. (2012), in which the reducing power of protein TCP-III increased with increase in concentration (6–30 μM). GTP, OTP and BTP showed linear correlations between concentration and reducing power with the coefficient R^2 of 0.9994, 0.9928, and 0.8994, respectively. There were significant difference between the reducing power of OTP, BTP and GTP (*p*<0.05). The reducing power of the three protein samples were in the orders of OTP > GTP > BTP, which was in agreement with the order on DPPH scavenging activity. The mechanism might be tea proteins, acting as electron donors and terminate radical chain reactions.

Inhibitory effects on lipid peroxidation induced by Fe^{2+} /ascorbate

Free radicals almost exist in bodies everywhere, if the dose was too high in lipid, lipid peroxidation would form, and

Fig. 2 SDS–PAGE of GTP, OTP and BTP. Lane 1: GTP, Lane 2: OTP, Lane 3: BTP, Lane 4: Protein molecular weight markers **Fig. 3** Antioxidant activities of GTP, OTP and BTP, **a** Scavenging effects (%) on 1,1-diphenyl-2-picrylhydrazyl; **b** Reducing power (absorbance at 700 nm); **c** Inhibitory effects on lipid peroxidation induced by Fe^{2+} /ascorbate

thus cellular damage would occur followed by more serious disease (Halliwell et al. 1992). Malondialdehyde (MDA), one of the products in lipid peroxidation, was widely used as a marker to test the degree of lipid peroxidation (Ardestani and Yazdanparast 2007). In this study, Fe^{2+} /ascorbate system was used to induce lipid peroxidation of rat liver. With the increase of concentration, the inhibitory rates of the tea protein increased. All the samples, GTP, OTP, and BTP expressed high inhibition on lipid peroxidation at high dose (660 $\mu\text{g/mL}$) with the inhibitory rates of 74.31 %, 73.86 %, and 72.20 %, respectively (Fig. 3c). The IC_{50} of three protein samples were 200.34, 17.25, and 192.06 $\mu\text{g/mL}$, respectively, for GTP, OTP and BTP. The results suggested that the

inhibition ability of three protein samples on lipid was in the decreasing order of OTP > GTP > BTP ($p < 0.05$).

According to the solubility, protein can be divided into four types of protein, including water-soluble, salt-soluble, alcohol-soluble and alkali-soluble proteins. In this study, the tea protein was dissolved in aqueous solution to detect the antioxidant activities. There was a positive relationship between the protein concentration and antioxidant activities, the higher protein concentration solution showed the higher DPPH scavenging activity, which proved the antioxidant activities of tea protein. The antioxidant capacity of salt-soluble, alcohol-soluble and alkali-soluble proteins will need further study. Among the three kinds of proteins, OTP exhibited the best antioxidant capacities in the three detected assays. OTP had the highest protein content compared with the other two. The results showed that soluble tea protein had good antioxidant activities, and there was a positive correlation between the content and activities.

Functional properties

Solubility

Solubility of varies protein samples extracted from green tea, oolong tea and black tea in the pH value range of 5.0–13.0 are shown in Fig. 4a. All the three protein samples exhibited weakest solubility at pH 5.0. The results indicated that the

Fig. 4 Solubility (a) and foaming (b) properties of GTP, OTP and BTP in different pH environments

isoelectric point of the three proteins were close to pH 5, which was in agreement with the other protein samples extracted from tea (Li et al. 2006; Wu et al. 2008) with the isoelectric point of 4.5. The solubility of GTP, OTP and BTP increased with the pH value increased. The maximum solubility were observed at pH 11.0 (97.03 % for GTP and 96.52 % for OTP) and at pH13.0 (84.11 % for BTP). The difference of the three proteins might owe to the different protein contents of the samples.

Water absorption capacity (WAC) and oil absorption capacity (OAC)

WAC and OAC are usually used to determine the amounts of polar and lipophilicity groups in the surface of the protein and their combination with H₂O and lipid molecules. In ordinary circumstances, the more polar groups there are in the surface of the protein molecules, the stronger the water absorption capacity it exhibits. The oil absorption capacity of the protein sample is influenced by the difference combination ability between lipophilicity surface of protein and hydroxyl groups of lipid. Among the three proteins, OTP exhibited the highest water absorption capacity (1.36 mL/g), while BTP showed the highest oil absorption capacity (1.58 mL/g) (Table 5. All the data obtained here were a little smaller than those of the previous reports on tea protein (Li et al. 2006; Wu et al. 2008), which might be due to the different resources and different processing of tea. There were significant difference between the WAC of GTP, OTP and BTP ($p < 0.05$). This might be due to the various conformational characteristics, surface hydrophobicity, intermolecular interactions, lipophilic groups and degeneration of the proteins (Deng et al. 2011) during production process of tea. In Chau' report, the viscosity of food system, as well as pH, ionic strength, temperature and concentration of the solution would also affect WAC and OAC (Chau et al. 1997). Compared with the results of OAC, WAC of tea protein was more positively related to protein solubility. The analysis of Aletor et al. (2002) demonstrated that water absorption capacity values ranging from 1.49 to 4.72 g/g could be used in viscous foods. It may be further studied to modify the tea protein samples to be a good choice of products requiring high water retention.

Emulsifying capacity (EC) and emulsifying stability (ES)

Protein can spread and be adsorbed on the oil-water interface, which would be one of the most important features to determine the emulsifying capacity and stability. The occurrence of emulsifying depends on the rapid absorption of the material, the hydrophilic-lipophilic balance, and ES is affected by the reduction of molecular surface energy and the rheological properties of membrane. The highest solubility

pH value was chosen to study the EC and ES of protein samples (Table 5). Through repeat experiments, the emulsifying capacity (EC) was GTP > BTP > OTP, but the emulsifying stability (ES) was opposite ($p < 0.05$). The results of ES illustrated that tea protein was hard to form emulsion.

Foaming capacity (FC)

Foaming capacity is one of the most important properties of protein application in food industry, which may be used in the production of ice-cream, cakes, and so on. In molecular level, the flexibility of the protein, this diffuses more rapidly to the air-water interface to encapsulate air particles and then enhances the foaming capacity (Chau et al. 1997). Moreover, the solubility of protein is also a critical factor of FC (Shen et al. 2008; Wu et al. 2008). Figure 3b showed that the higher protein solubility implied the better foaming capacity. There was a similar trend in the FC analysis of OTP and BTP. However, there was a small decreasing of GTP in the pH ranging from 7.0 to 11.0 then a sharp increasing at pH 13.0. FC of GTP, OTP and BTP varied from 25.13 % to 29.57 %, from 27.83 % to 43.33 % and from 20.50 % to 40.60 %, respectively. The highest FC values of GTP and BTP were at pH 13.0, while FC of OTP reached the highest value at pH 11.0. There was a significant difference between GTP and OTP ($p < 0.05$). Compared with the results of other protein from plant sources, such as peanut protein (Wu et al. 2008) and *Ginkgo biloba* protein (Deng et al. 2011), there would be a superiority for tea protein to be food supplement in large concentration.

Gelating capacity (GC)

Gelating capacity played a main role in many food preparations. The formation of protein gel is impacted by the following factors, such as solution concentration, stirring speed, temperature, heating time, cooling time, which are essential to gelatinization. Gel formed by cooling is relied on the association of hydrogen bonding and hydrophobic interactions, because these two bindings easily formed and re-folding of partial protein came up at lower temperatures. With the same minimum gelling concentration (60 mg/mL), tea protein exhibited good GC and could be applied in the manufacture of all kinds of dairy products, such as jelly, condensation protein, gelatin gel, bread.

Conclusion

In this study, alkali technology was employed to extract protein from tea and optimized by Respond Surface Method (RSM). Box-Behnken design (BBD) was used to determine the optimum process parameters that could give a high

extraction yield. The optimal conditions determined are as follows: extraction time of 85 min, extraction temperature at 80 °C, alkali concentration of 0.15 M. Under these optimal conditions, a maximum protein yield (20.73 %) was achieved. Furthermore, the antioxidant activities and functional properties of protein extracted under the optimum conditions from three different kinds of tea (green tea, oolong tea and black tea) were comparatively studied and oolong tea protein had better antioxidant activities and functional properties with higher protein contents 527.67 mg/g. Oolong tea protein might be used in the food industry.

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