Optimizing and predicting degree of hydrolysis of ultrasound assisted sodium hydroxide extraction of protein from tea (*Camellia sinensis* L.) residue using response surface methodology

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Abstract Response surface methodology was employed to investigate the effect of sodium hydroxide concentration (X_1 : 0.05–0.15 M), sonication time (X_2 : 5–15 min), ultrasonic power (X_3 : 150–450 W/L), and solid–liquid ratio (X_4 : 20–60 g/L) on the optimization of protein extraction from tea residue. Single frequency countercurrent ultrasound (SFCU) was employed to assist the extraction and subsequent hydrolysis of the protein. Optimal extraction conditions were established and response surfaces were generated using mathematical models. There were positive linear and negative quadratic effects of extraction variables on protein yield. The optimal predicted protein yield of 138.9 mg/g was obtained under the optimum conditions of concentration of 0.13 M, extraction time of 13 min, ultrasonic power of 377 W/L and solid–liquid ratio of 51.5 g/L. A model for the degree of hydrolysis of the extraction process was also obtained which gave a predicted and experimental value of 8.4% and 7.5% respectively. Essential amino acid content of 36.7% was obtained under optimal conditions.

Keywords Box–Behnken · Sonication · Amino acids · Hydrolysis · Alkali

Introduction

Tea (*Camellia sinensis* L.) has high patronage and consumption around the world for which reason a lot of scientific research have been geared towards unraveling its composition and pharmaceutical functions (Savić et al. 2014; Shi et al. 2011). Most of the research, however, is centered on the polyphenols and catechins because of their many acclaimed health benefits including biological activities like antioxidant effects (Fukushima et al. 2009), anticarcinogenic (Yang et al. 2009) and antitumor potentials (Li et al. 2008).

Being a major agricultural product in China, about 2.26 million tons of tea leaf products are produced annually of which 20% accounts for waste generation in the form of residue (Zhang et al. 2014). Various processing techniques, however, have been adopted over the years in the production process leading to variations in tea products from the same plant. These variations in processing have effects on the sensory perceptibility of these teas with a likely effect on the final residue generated as waste or by-product. The residue mainly leaves and twigs generated after infusion promises to be a potential source of protein (Zhang et al. 2014).

For decades now, protein from unconventional plant sources have found application as an additional protein source (Ghaly and Alkoaik 2010). These proteins have the potential of being used in animal feed (Kammes et al. 2011) or food production (Ghaly and Alkoaik 2010) or could be hydrolyzed to amino acids or peptides for other applications. These could include commodity chemicals

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(Gallezot 2012) and other bioactive compounds in drug formulations. Meanwhile, the major limitation in leaf protein extraction has to do with the low levels of extractable proteins ranging between 15 and 60% (Chiesa and Gnansounou 2011).

The usage of alkali in protein extraction has been studied for some time now (Shen et al. 2008; Chittapalo and Noomhorm 2009; Zhang et al. 2014). Shen et al. (2008) demonstrated the effectiveness of alkali extraction compared with enzyme assistance and extraction, of tea protein. Though alkali extraction resulted in high protein yield compared with enzymes as indicated, the yield was still relatively low. Zhang et al. (2014) intimated that this barrier in alkali extraction can be ameliorated by thermal assistance. Meanwhile, using high temperature in thermal assistance is not favorable in protein extraction, for it may decrease protein yield because of considerable coagulation (Csapó et al. 2008) and increase protein hydrolysis (Bals and Dale 2011). This may also reduce protein quality as a result of denaturation, hydrolysis, or amino acid racemization (Bals and Dale 2011; Csapó et al. 2008).

This effect of temperature could be ameliorated if ultrasonic-assisted extraction (UAE) is employed. UAE may prove useful in this regard as it has been widely employed as a pretreatment step in the extraction of valuable biomass components attributable to its high efficiency and low operating cost compared to the traditional extraction methods (Roldán-Gutiérrez et al. 2008). It has been applied in the extraction of biological molecules from different plant materials (Khan et al. 2010; Ghafoor et al. 2009). Yang and Zhang (2008) used it as a low-cost alternative to solvent reflux extraction of phenolic compounds from plants. That notwithstanding, its applicability in alkali extraction with reference to brown tea residue has not been studied. There is therefore the need to adopt the use of ultrasound in alkali extraction and to optimize protein extraction process using surface response methodology. In furtherance to this, there is also the need to investigate the effect of the extraction conditions on the quality of the extracted protein by considering the effect on the degree of hydrolysis.

The response surface method has many designs for it and has been used in many optimization studies. According to Ferreira et al. (2007), the Box Behnken design (BBD) compares favorably to other response surface designs such as central composite and three-level full factorial design. It has been verified that the BBD and Doehlert matrix are slightly more robust than the central composite design (Ferreira et al. 2007). This is attributable to the fact that it simultaneously contains combinations of factors which are randomly at the highest, lowest and central points in the design, hence useful in avoiding experiments performed under extreme conditions, for which substandard results

might occur (Ferreira et al. 2007). By varying extraction parameters such as sodium hydroxide concentration, solid–liquid ratio, ultrasonic power and time, the maximum extractable yield of protein from tea residue could be obtained. The objective of this paper, therefore, was to optimize ultrasound-assisted alkali extraction of brown tea residue using Box–Behnken optimization methodology and further use the technique to predict the degree of hydrolysis of the extracted proteins and determine its amino acid composition.

Materials and methods

Mashed brown tea residue used for protein extraction was obtained from Nanjing Apogee Food Technology Company Limited, Nanjing. Sodium hydroxide (NaOH), *O*-phthalaldehyde (OPA), dithiothreitol, Sodium dodecyl sulphate (SDS), sodium tetraborate decahydrate ($\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$), Bovine serum albumin, serine, Coomassie Brilliant blue G-250 were purchased from Hangzhou Xiaoshan Chemicals (Hangzhou, China). Ammonium sulphate, Phosphoric acid and methanol were obtained from Tianjin Zhiyuan Chemical Reagent Co. Ltd. (Tianjin, China). All chemicals used in this work were of analytical grade.

Determination of crude protein in tea residue

Kjeldahl method was used to determine the crude protein in the residue. Determinations were in triplicate and the average used in the analysis (AOAC 1990).

Sample pretreatment

Brown tea residue with protein content 21.1 g/100 g ($N \times 6.25$) was dried in an oven at 50 °C for 24 h. The residue was sieved with an 80 µm mesh before extraction. Respective grams of tea residue and NaOH concentration (0.05, 0.1 or 0.15 M) were added to give a solid–liquid ratio of 20 g/L, 40 g/L and 60 g/L. The mixture was then subjected to ultrasound treatment in a reconstructed single frequency countercurrent ultrasound processor, which was equipped with a 2 cm flat probe (Fanbo Biological Engineering Co., Ltd., Wuxi, China; Model FBTQ 2000) (20 kHz with circulating pump speed of 300 r/min and pulsed on-time and off-time of 3 and 2 s.) for 5 min, 10 min and 15 min with varying power output of 150 W/L, 300 W/L and 450 W/L. The effect of temperature on the alkali extraction was verified under similar condition but without sonication at varying temperature of 25, 40 and 60 °C for 30, 60, 90 and 120 min. The extracted proteins

were then subjected to similar analysis. After extraction, the pH was rapidly adjusted to 7.0 using drops of 1 M HCl.

Purification of tea protein

After extraction, samples were centrifuged at 5000g and the resultant raw protein solution saturated with 65% ammonia sulphate. The precipitated solution was also centrifuged at 5000g and the collected protein after being re-solubilized with 10 mM phosphate buffer saline was dialyzed with distilled water.

Soluble protein

The soluble protein was measured according to the method of Bradford (1976). 100 μ L of the extracted protein sample was measured and placed in a test-tube. Five mL of Coomassie Brilliant Blue solution was added and the resulting mixture was vortexed. Bovine serum albumin (BSA) (0–1000 μ g/mL) was prepared and used as the standard. The absorbance of the samples and standard were measured at a wavelength of 595 nm on a UV–Vis spectrophotometer, model UV-1000 (Shanghai, China).

Degree of hydrolysis

The method of Nielsen et al. (2001) was used. 7.620 g of di-sodium tetraborate decahydrate ($\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$) and 200 mg sodium-dodecyl-sulfate (SDS) were completely dissolved in 150 mL deionized water. 160 mg *o*-phthalaldehyde 97% (OPA) was also dissolved in 4 mL ethanol. The OPA solution was then transferred to the di-sodium tetraborate decahydrate and SDS solution with deionized water. 176 mg dithiothreitol 99% (DTT) was then added to the solution by rinsing with deionized water. The mixture was made up to 200 mL with deionized water. Serine standard was prepared by dissolving 50 mg of serine in 500 mL of deionized water (0.9516 meqv/L).

The sample solution was prepared by dissolving 0.5 g sample (Freeze dried samples after dialysis) in 100 mL distilled water. Three mL OPA reagents were added to all test tubes. Four (4) test tubes were used for standard, 4 for blank, and four for each sample. Because absorbance may change with time the samples were made to stand for exactly 2 min before readings were made. The assay was carried out at room temperature of 25 °C. 400 μ L serine standard was added to a test tube containing 3 mL OPA reagents and mixed for 5 s. The mixture was made to stand for exactly 2 min before the absorbance was read at 340 nm. The absorbance of two of the standards was taken before the blanks along with sample values. The absorbance of the other 2 standards were determined after

having taken all blanks and sample values. The mean of these values was used in the calculation.

Amino acid composition

The total amino acid composition of the alkali extracted and ultrasound-assisted extracted proteins was determined by hydrolyzing the samples with 6.0 M HCl in sealed glass tubes at 110 °C for 24 h. The samples were then filtered and made up to 50 mL with distilled water. Aliquots (1.0 mL) of diluted samples were filtered with 0.22 μ m membrane and analyzed by amino acid analyzer (RP-HPLC, Agilent Technologies, Woburn, MA, USA) according to Li et al. (2016).

Box–Behnken design

Single factor experiments were initially conducted and according to the results, four factors of NaOH concentration, sonication time, ultrasound power and solid–liquid ratio were selected. Box–Behnken response surface design of the four factors and three levels were used and + 1, 0, – 1 encoded factors were made to represent the selected variables. The independent variables X_i , which were defined as dimensionless, was coded as x_i , according to the Eq. (1)

$$x_i = \frac{(X_i - X_0)}{\Delta X_i} \quad (1)$$

where x_i is the coded value of an independent variable, X_i represents the actual value of the independent variable, X_0 is the actual value of the independent variable at the center of the domain and ΔX_i is the respective 1 unit increment of X_i . The Box–Behnken design comprised 27 experimental points including 3 central points based on the recommendation of the Minitab 17 software (Tables 1, 2).

Statistical analysis

All experiments were performed in triplicate and the average used in the analysis. Data was subjected to one-

Table 1 Box–Behnken experimental design factors and levels of encoded values

Factors	Symbols		Levels		
	Coded	Uncoded	– 1	0	+ 1
NaOH concentration	x_1	X_1	0.05	0.10	0.15
Time (min)	x_2	X_2	5	10	15
Ultrasound power (W/L)	x_3	X_3	150	300	450
Solid liquid ratio (g/L)	x_4	X_4	20	40	60

Table 2 Box–Behnken design and experimental results

Number	χ_1	χ_2	χ_3	χ_4	Yield (mg/g)	DH (%)
1	0	0	0	0	130	4.39
2	−1	−1	0	0	64	3.29
3	0	−1	1	0	88	6.95
4	−1	0	0	−1	62	3.66
5	0	1	0	1	127	7.20
6	1	0	−1	0	100	7.56
7	0	1	0	−1	83	4.27
8	1	−1	0	0	97	6.59
9	0	0	−1	−1	74	4.51
10	0	1	−1	0	82	4.88
11	−1	1	0	0	67	3.17
12	0	−1	0	1	79	4.88
13	−1	0	0	1	67	4.63
14	1	0	1	0	117	9.02
15	1	0	0	1	114	7.80
16	0	0	1	1	119	9.39
17	1	0	0	−1	102	6.59
18	−1	0	−1	0	67	3.90
19	0	−1	0	−1	91	5.85
20	−1	0	1	0	74	5.37
21	0	−1	−1	0	77	4.51
22	0	0	0	0	126	5.12
23	0	0	1	−1	102	6.83
24	0	0	0	0	121	5.37
25	1	1	0	0	119	9.15
26	0	0	−1	1	94	4.63
27	0	1	1	0	117	6.83

way analysis of variance (ANOVA). LSD's test was used to ascertain the differences ($p < 0.05$) in means of yield between thermal and ultrasound-assisted sodium hydroxide extraction as well as their effect on the degree of hydrolysis using Minitab 17.1.0.0 (Minitab Inc., State College, PA, USA, 2013).

Results and discussion

Effect of extraction parameters on protein yield

Preliminary investigations considered the effect of various parameters such as ultrasound frequency, power, different alkali (NaOH and Ca (OH)₂) and extraction time on extraction of protein from brown tea residue. A significant ($p < 0.5$) relationship existed between NaOH concentration, Ultrasound power, sonication time and solid–liquid ratio in protein extraction and these parameters were thus

chosen in the optimization process. In finding the optimum conditions for ultrasound-assisted NaOH protein extraction, response surface using Box–Behnken design was used to fit regression models to the experimental data. The respective model for the real variables was given as follows:

$$\begin{aligned}
 \text{Yield} \left(\frac{\text{mg}}{\text{g}} \right) = & -138.3 + 1930X_1 + 6.42X_2 \\
 & + 0.3583X_3 + 1.733X_4 - 9150X_1^2 \\
 & - 0.7100X_2^2 - 0.000656X_3^2 - 0.03594X_4^2 \\
 & + 15.0X_1X_2 + 0.333X_1X_3 + 1.75X_1X_4 \\
 & + 0.00800X_2X_3 + 0.1400X_2X_4 - 0.000250X_3X_4
 \end{aligned} \quad (2)$$

Ultrasound-assisted NaOH extraction from brown tea residue had higher extraction yield 134 ± 2.8 mg/g (63.5%) compared with the use of only NaOH (56.4%), and NaOH and enzyme assisted extraction (47.8%) from tea residue in previous studies (Shen et al. 2008). Chittapalo and Nookhorm (2009) in extraction of protein from defatted rice bran using ultrasonic assistance recorded a higher yield and improved coloration than the conventional method. The relatively high extraction rate observed in the study could be attributed to sonication. The ultrasound-assisted extraction mechanism involved cavitation generated in the extraction mixture by the passage of ultrasonic waves leading to the diffusion of extractable protein through the tea residue matrix (Chemat et al. 2017). Thus, ultrasonic waves generated forces perpendicular to extractable components resulting in shear strain leading to an increase in mass transfer of extractable components (Zou et al. 2013). In addition to this, the collapse of empty bubbles in the tea residue mixture generated chaotic changes in pressure and flow velocity. This might have led to high-velocity resulting in intensive collision within the mixture. This is likely to have resulted in re-orienting the tiny particular pores of the tea residue which accelerated the mixture of proteins from the residue to the extraction solvent due to eddy motion and internal diffusion resulting in surface abrasion, dissolution and tea residue breakdown (Vilkhu et al. 2008). The use of NaOH also had a major role to play in the extraction process. Objectively, substantial breakdown of tea residue was observed to have occurred. Prior to sonication, sodium hydroxide had changed the physical orientation of the tea residue by possibly dissolving the glutelin fraction of proteins in the residue. This was corroborated by the fact that various extraction solvents and buffer used prior to sonication had yield that was significantly ($p < 0.05$) low. A substantial quantity of 36.5% of protein still remained trapped. This might be because proteins in original pulp of tea had been extracted in the tea manufacturing process with some of the

remaining protein being of low solubility. Conversely, large protein molecules may have been hydrolyzed to smaller peptides and amino acids following sonication and extraction with sodium hydroxide. The colour of Coomassie Brilliant Blue G-250 will switch from red to blue when binding with proteins and the absorbance can be measured at 595 nm (Bradford 1976). The Bradford protocol, however, can only detect proteins that have molecular weight more than 3000 Dalton and thus smaller proteins, peptides and amino acids may not have been detected after the extraction process (Grintzalis et al. 2015).

Fitting of response variable during optimization may be misrepresented unless the model exhibits an adequate fit (Tang et al. 2010). It was therefore pertinent to check the adequacy of the model. The use of analysis of variance (ANOVA) gave the validity of the model and explained whether the model adequately fitted the variations observed in protein yield extracted at the designed extraction level. A major parameter in determining the fit of a model is the *F* test. For a model to be useful, the *F* test for the model must be significant at the 5% level ($p < 0.05$). The implication was that the model fitted and could adequately explain the variations observed. However, if the *F* test for the lack of fit is significant ($p < 0.05$) then a more complex model is required to model the data (Tang et al. 2010) (See supplementary data). The coefficient of determination (R^2) of the predicted model was 97.63%. This suggested that 97.63% of the variations in the extraction parameters could be explained by the fitted model. Therefore, the model sufficiently represented the actual correlation between the parameters chosen. R^2 value greater than 80% for a model has been deemed adequate by researchers (Zou et al. 2013; Qu et al. 2010). The regression model was in good agreement with the experimental results. A comparison of the observed and predicted values respectively from experimental and calculated suggested that the models used in the research were able to identify optimum operating conditions for the protein extraction.

The influence of NaOH concentration, ultrasonic time, ultrasonic power and solid–liquid ratio on the extraction yield was significant ($p < 0.05$). However, ultrasonic power had the greatest effect followed by solid to liquid ratio, concentration and ultrasonic time. The effect of sonication is greatly seen in the intensity of the waves generated and the duration at which the intensity lasts. All the terms were significant except X_1X_2 , X_1X_3 , X_1X_4 and X_3X_4 indicating that the effect of the interactions between the factors was not significant ($p < 0.05$). The model had a positive linear and a negative quadratic effect of extraction variables on protein yield. The protein extraction yield increased as the levels of these factors increased, and decreased as the levels of increased above a certain threshold. Thus continual increase in extraction parameters

could only result in increase in extraction up to a certain optimum after which it will have a negative effect on the protein yield as was observed in the study. In order to appreciate the response surfaces of ultrasound-assisted alkali extraction parameters, Fig. 1 shows the interaction of every two factors. (See supplementary data for ANOVA for response surface quadratic model for Yield of protein).

Validation of model for protein extraction

The optimum conditions of protein extraction for concentration, ultrasonic time, ultrasound power and solid–liquid ratio were obtained from the generated model. To test the validity of the model, protein was extracted under the optimal conditions and the protein yield was determined. An amount of 134 ± 2.8 mg/g ($n = 3$) of protein was obtained in a control experiment carried out under the optimized operating condition (concentration of 0.13 M, ultrasonication time of 13 min, ultrasonic power of 377 W/L and solid–liquid ratio of 51.5 g/L). The experimental yield of protein was in consonance with the predicted value.

Effect of extraction parameters on degree of hydrolysis

Response surface regression analysis was further carried out after optimizing protein extraction to model and predict the effect of extraction parameters, thus concentration of NaOH, ultrasound power, sonication time and the solid–liquid ratio on the degree of hydrolysis. The predictive model for uncoded units was given as follows:

$$\begin{aligned} DH(\%) = & 13.33 - 28.9X_1 - 0.610X_2 - 0.0232X_3 \\ & - 0.2175X_4 + 187X_1^2 + 0.0028X_2^2 \\ & + 0.000041X_3^2 + 0.001016X_4^2 + 2.68X_1X_2 \\ & + 0.61X_1X_4 - 0.000163X_2X_3 + 0.00976X_2X_4 \\ & + 0.000203X_3X_4 \end{aligned} \quad (3)$$

(See supplementary data for the ANOVA for the predictive model for degree of hydrolysis). The coefficient of determination which is R^2 gave the ratio of explained disparity to the total variation. It, therefore, estimated the fitness of the model. For a model to fit well, the R^2 must approach 100%. A small R^2 indicates a poor response to the model. The coefficient of determination (R^2) of the predictive model was 93.85%, suggesting that 93.85% of the variations could be explained by the fitted model. Meanwhile, a larger R^2 does not always validate a good model. For a statistically good model, the adjusted R^2 should be close to R^2 . The adjusted R^2 was 86.68%.

Fig. 1 Surface plots for brown tea residue protein extraction. **a** Figure plot to show time (min) and solid–liquid ratio (g/L). **b** Figure plot to show concentration (M) and time (min). **c** Figure plot to show concentration (M) and ultrasound power (W/L). **d** Figure plot to show concentration (M) and solid–liquid ratio (g/L). **e** Figure plot to show time (min) and ultrasound power (W/L). **f** Figure plot to show ultrasound power (W/L) and solid–liquid ratio (g/L)

Therefore, the model adequately represented the real relationship between the parameters chosen. The model (Eq. 3) gave a significant negative linear relationship and a positive insignificant quadratic relationship. This observation indicated that the degree of hydrolysis would relatively change with subsequent change in any of the extraction conditions. Thus, an increase in degree of hydrolysis would be observed with an increase in any of the extraction conditions depending on their effect on the predictive model. As it was observed, the degree of hydrolysis increased with increase in concentration, ultrasound power and solid–liquid ratio. The effect of these three parameters on the degree of hydrolysis was significant ($p < 0.05$). Jambrak et al. (2008) have reported associated findings when whey protein samples were sonicated. The increase in degree of hydrolysis with increase in ultrasound power and solid–liquid ratio can be attributed to enhanced cavitation effect resulting from the presence of more cavitationally active volume with time (Deenu et al. 2013). Sodium hydroxide also has the ability to decompose proteins at ambient temperature resulting in the observed effect on the degree of hydrolysis after being used in combination with sonication.

To calculate DH, it was necessary to have values for h and h_{tot} according to the equation $\text{DH} = 100 h/h_{\text{tot}}$. The expression for h in the OPA method was given by $h = (\text{serine-NH}_2 - \beta)/\text{amegv/g protein}$. α , β (Nielsen et al. 2001) and h_{tot} (Adler-Nissen 1987) values of 0.4, 1.0 and 8.2 were respectively used in the calculation of the degree of hydrolysis. The use of OPA method in determining the degree of hydrolysis has proven effective than other methods in other studies (Morais et al. 2013; Nielsen et al. 2001). The values herein obtained indicated that relatively minimal degree of hydrolysis occurred during the extraction.

Validation of the model for degree of hydrolysis

The model was validated when the degree of hydrolysis at the optimum extraction condition (concentration, 0.13 M; time, 13 min, power, 377 W/L and solid–liquid ratio of 1 g: 45.7 mL) was determined. The predicted degree of hydrolysis according to the model was $8.4\% \pm 0.16$ and the experimentally determined degree of hydrolysis was $7.5\% \pm 0.12$.

Effect of thermally assisted alkali extraction on protein yield and degree of hydrolysis

Analysis of variance was conducted by studying the effect of temperature and time on the protein extraction yield of brown tea residue using alkali. A significant relationship ($p < 0.05$) existed between the effect of alkali concentration and time on the protein yield. The maximum extraction yield of 79 mg/g was obtained at an alkali concentration of 0.15, a time of 120 min and temperature of 60 °C. This extraction rate represented 41.04% of ultrasound alkali assisted extraction process. The implication was that substantial reduction in time followed by a relatively high extraction of protein had been achieved following the ultrasound-assisted optimization procedure. Increase in NaOH concentration coupled with the increase in temperature may result in increase in protein yield but at a high time duration. A high hydrolysis percentage is also expected at such high increase in thermal assistance. As indicated in other studies thermally assisted alkali extraction does not in most cases lead to high extraction because of protein coagulation, denaturation or hydrolysis (Bals and Dale 2011; Csapó et al. 2008). Further analysis of the effect of alkali extraction at a temperature of 60 °C also indicated that alkali concentration had a significant effect ($p < 0.05$) on the degree of hydrolysis. Variation of time did not have any significant effect ($p > 0.05$) on the degree of hydrolysis following the thermal treatment.

Amino acid composition

Comparison between amino acid composition from thermally assisted and ultrasound-assisted extracted proteins from this work and other studies are presented in Table 3. The amino acid composition of the ultrasound-assisted extracted protein from brown tea residue (BTR) was slightly lower than that obtained by Zhang et al. (2014) after extraction of proteins from green tea residue at 95 °C. This might be attributed to variations in the processing of these tea varieties leading to variations in their residue. However, ultrasonication has minimized the extraction time significantly. The use of alkali and elevated temperature might have also resulted in the generation of lysinoalanine through intermolecular cross-coupling and rearrangement of proteins (Hou et al. 2017) with Lys and Cys contents being greatly affected. This might have led to reduction in Lys and Cys contents in proteins extracted at 60 °C and 95 °C (Table 3). Essential amino acids being arginine, histidine, leucine, isoleucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine are of prime importance to humans and monogastric animal. The essential amino acid content of 36.7% and 28.3% was obtained from ultrasound-assisted and thermally assisted

Table 3 Amino acids composition in tea protein (g/100 g protein)

Amino acid	60 °C alkali extraction	UAE extract BTR	^a 95 °C GTR extract	^b GTR
Asp	7.47 ± 0.12	10.70 ± 0.10	11.6 ± 0.7	8.0
Thr	3.40 ± 0.17	3.60 ± 0.10	3.1 ± 0.2	3.8
Ser	3.43 ± 0.12	4.07 ± 0.06	4.3 ± 0.1	3.9
Glu	8.20 ± 0.10	12.57 ± 0.06	12.4 ± 0.5	10.1
Gly	3.87 ± 0.06	5.37 ± 0.06	5.9 ± 0.2	4.6
Cys	0.05 ± 0.03	0.09 ± 0.01	–	0.8
Val	3.97 ± 0.06	5.80 ± 0.17	5.5 ± 0.2	4.6
Met	0.99 ± 0.01	1.03 ± 0.06	1.9 ± 0.1	1.1
Ile	3.17 ± 0.06	5.73 ± 0.12	6.3 ± 0.3	4.0
Tyr	0.70 ± 0.17	0.97 ± 0.06	4.3 ± 0.1	–
Phe	4.10 ± 0.10	5.03 ± 0.12	4.4 ± 0.2	4.5
lys	1.97 ± 0.06	5.07 ± 0.06	2.8 ± 0.2	5.6
His	0.92 ± 0.03	1.48 ± 0.08	1.9 ± 0.1	2.0
Arg	4.33 ± 0.06	4.63 ± 0.12	3.4 ± 0.2	4.8
Pro	3.13 ± 0.06	4.47 ± 0.06	4.8 ± 0.2	3.6
Leu	7.45 ± 0.05	9.37 ± 0.06	9.0 ± 0.3	7.8
Ala	3.37 ± 0.12	6.60 ± 0.14	6.0 ± 0.1	4.9
TAA	60.51 ± 1.36	86.58 ± 1.40	87.7 ± 2.1	77.0
HAA	28.93 ± 0.66	39.09 ± 0.8	42.2 ± 1.5	31.3
SCAA	1.04 ± 0.07	1.12 ± 0.07	1.9 ± 0.1	1.9
AAA	4.80 ± 0.27	6.0 ± 0.18	8.7 ± 0.4	4.5
PCAA	7.22 ± 0.15	11.18 ± 0.26	8.1 ± 0.5	12.4
NCAA	15.67 ± 0.20	23.27 ± 0.16	24.0 ± 1.2	18.1

^aZhang et al. (2014), ^bShen et al. (2008)

TAA total amino acids, HAA hydrophobic amino acids (Met, Phe, Val, Leu, Ile, Pro, Ala, Cys, Tyr), SCAA sulfur-containing amino acids (Met, Cys), AAA aromatic amino acids (Phe, Tyr), PCAA positively charged amino acids (Arg, His, Lys), NCAA negatively charged amino acids (Asp, Glu)

alkali extraction respectively. The amino acid composition of BTR at the optimum extraction rate is also comparable to soy proteins in other studies (Frikha et al. 2012).

Conclusion

Response surface methodology was successfully used to optimize protein extraction from brown tea residue. Four parameters comprising sodium hydroxide concentration, ultrasonication time, ultrasound power and solid–liquid ratio were considered using the Box–Behnken design. Protein yield of 134.8 mg/g (63.5%) was obtained under the optimum conditions of 0.13 M NaOH concentration, 13 min sonication time, 377 W/L ultrasonic power and 51.5 g/L solid–liquid ratio. The experimental yield agreed closely with the predicted yield under optimized conditions. The predicted degree of hydrolysis also agreed closely with the experimental value when the degree of hydrolysis was modeled under the extraction conditions. The amino acid composition of the ultrasound-assisted NaOH extracted proteins at the optimum conditions

compared favorably to proteins extracted with heat assistance in the study.

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