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Amino acid composition, molecular weight distribution and antioxidant activity of protein hydrolysates of soy sauce lees

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ABSTRACT

The proteins of soy sauce lees (SSLP) were hydrolysed by Alcalase in the presence of ultrasound or traditional water bath to obtain hydrolysates S2–S6. The analysis of protein content indicated that enzymatic hydrolysis could significantly improve the extraction efficiency of proteins. By determination of molecular weight distribution, >10 and 5–10 KDa fractions of native SSLP (S1) decreased during hydrolysis, whilst 3–5 KDa fraction increased. Gradual increases of free, total and antioxidant amino acids were observed for S1–S4, and the differences between S4 and S6 were slight. Tyrosine was the major free amino acid of S1–S6, whilst glutamic acid had the highest amount in total amino acid composition. S2–S6 showed stronger DPPH radical scavenging activities in a dose-dependent manner than S1. All the results suggested that ultrasound treatment showed an inhibition behaviour on the enzymatic hydrolysis of SSLP.

1. Introduction

Soy sauce is a traditional seasoning in China and many other Asian countries (Luo, Ding, Chen, & Wan, 2009). It is essential for improving flavour and taste of food. The salty taste and sharp flavour makes it more and more popular all over the world (van der Sluis, Tramper, & Wijffels, 2001). Moreover, soy sauce contains various bioactive components, which have been reported to possess anticarcinogenic, antimicrobial, antiplatelet and immunomodulating activities (Kobayashi, 2005). Soy sauce lees, the main by-product of the soy sauce process, are precipitated during the refining programme. They account for 5–10% of the whole volume of raw soy sauce (Furukawa, Kokubo, Nakamura, & Matsumoto, 2008). Preliminary work has found that proteins and carbohydrates are the main components of soy sauce lees. The protein fraction accounts for approximate 20% of the total weight. It confirms soy sauce lees as a good source of proteins. Due to the special structure of these proteins and covalent linkage to other components of plant tissues, they are difficult to be hydrolysed by microorganisms during the sauce fermentation. Therefore, it is of significance to find a way to efficiently utilise this protein source.

As a proteolytic enzyme with broad specificity and strong hydrolysis capability, Alcalase has been used for hydrolysing vegetable proteins (Cui, Zhou, Zhao, & Yang, 2009). Through cleaving peptide linkage, enzymatic hydrolysis can decrease the molecular weight and enhance the functional properties of proteins (Klompong, Benjakul, Kantachote, & Shahidi, 2007). Furthermore, it can also facilitate the dissolution of proteins from plant material by breaking the linkage between proteins and other components. Ultrasonic wave is a novel technique for isolating macromolecules from plant materials in recent years. Its mechanical effect can facilitate the mass transfer of extractive solutes and improve the extraction efficiency (Yang, Jiang, Zhao, Shi, & Wang, 2008).

Application of enzymatic hydrolysis in combination with ultrasound treatment to prepare SSLP hydrolysates will be an interesting attempt to utilise this protein source. However, up to now, there are limited literature reports concerning this topic. Therefore, the objective of this work was to hydrolyse SSLP by Alcalase in combination with traditional water bath or ultrasound treatment. The amino acid composition, molecular weight distribution and antioxidant activity of SSLP hydrolysates were further investigated.

2. Materials and methods

2.1. Materials and chemicals

The soy sauce lees were donated by Guangdong Meiweixian Condiment Co., Ltd. (Zhongshan, Guangdong, China). The moisture content was determined to be 15.6 ± 0.6% (w/w) and protein content was 19.5 ± 1.1% (w/w).

Alcalase 2.4 l, with a nominal activity of 2.4 AU/g, was obtained from Novo Nordisk (Beijing, China). DPPH (1,1-Diphenyl-2-picrylhydrazyl) and amino acid standards were purchased from Sigma

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Co. (St. Louis, MO, USA). All the other chemicals used were of analytical grade.

2.2. Preparation of SSLP hydrolysates

Fifty grammes of soy sauce lees were mixed with 250 ml of deionized water in a water bath shaker (New Brunswick Scientifics C24, Jintan, China) at 50 °C for 30 min. The pH value was regulated to 8.5. Then 0.5 ml of Alcalase 2.4 l was added to initiate the hydrolysis for 10, 15, 30 and 60 min, respectively. The enzyme was inactivated by incubating in boiled water for 15 min, then centrifuged at 10,000*g* for 20 min. The supernatants were collected as S3 (hydrolysis for 10 min), S4 (hydrolysis for 15 min), S5 (hydrolysis for 30 min) and S6 (hydrolysis for 60 min).

The soy sauce lees slurry at pH 8.5 was kept in water bath shaker for 30 min at 50 °C without addition of Alcalase. Then it was subjected to the above programme as S3–S6. The supernatant was collected as the native SSLP (S1). Ultrasound treatment was taken to assist the enzymatic hydrolysis of SSLP. The slurry at pH 8.5 was kept in a ultrasonic cleaner. After adding 0.5 ml of Alcalase, the treatment was started at 120 W and 50 °C for 30 min. Then the enzyme was inactivated and subjected to above programme as S3–S6. The supernatant was collected as S2. S1–S6 were volumerised up to 200 ml by adding distilled water for protein quantification. Moreover, an aliquot (20 ml) of each sample was mixed with 20 ml of 20% (w/v) trichloroacetic acid (TCA) at room temperature (16 °C) for 30 min. Then it was centrifuged at 5000*g* for 20 min. The supernatant was collected to determine the TCA-soluble protein content. The nitrogen content was determined by Shimadzu 4100 Series Total Nitrogen Analyser (Shimadzu, Kyoto, Japan), and expressed as mg/ml. The protein content was calculated as 6.25 × nitrogen content.

2.3. Determination of molecular weight

Molecular weight distribution of S1–S6 were determined by gel permeation chromatography on a Superdex Peptide HR 10/300 GL (10 × 300 mm, Amersham Biosciences Co., Piscataway, NJ) with UV detection at 214 and 280 nm. The mobile phase (isocratic elution) was 0.02 M phosphate buffer containing 0.25 M NaCl (pH 7.2), at a flow rate of 0.5 ml/min. A molecular weight calibration curve was prepared from the average elution volume of the following standards: Cytochrome C (12,500 Da), aprotinin (6500 Da), vitamin B_{12} (1355 Da), oxidised glutathione (612 Da) and glycylglycylglycine (189 Da) (Sigma Co., USA). UNICORN 5.0 software (Amersham Biosciences Co., Piscataway, NJ) was used to analyse the chromatographic data.

2.4. Amino acid analysis

The amino acid composition of S1–S6 was determined according to the method of Sun et al. (2010) with a slight modification. Amino acid composition was determined by high performance liquid chromatography (Waters, Milford, MA) equipped with a PICO.TAG column. Free amino acid composition was determined by injecting S1–S6 directly into chromatography system. The total amino acid composition of S1–S6 were determined after hydrolysis at 110 °C for 24 h with 6 M hydrochloric acid prior to the derivatization with phenyl isothiocyanate. Alkaline hydrolysis at 105 °C for 24 h with 4 M NaOH was also done for determination of tryptophan (Trp) level. External standards were used for quantification. The amino acid standards included L-alanine (Ala), L-arginine (Arg), L-aspartic acid (Asp), L-cystine (Cys), L-glutamic acid (Glu), L-glycine (Gly), L-histidine (His), L-isoleucine (Ile), L-leucine (Leu), L-lysine (Lys), L-methionine (Met), L-phenylalanine (Phe), L-proline (Pro), L-serine (Ser), L-threonine (Thr), L-tyrosine (Tyr), L-valine (Val), L-tryptophan and ammonium chloride.

2.5. Assay of DPPH radical scavenging activity

The DPPH radical scavenging activity was measured by the method of Yang, Zhao, Prasad, Jiang, and Jiang (2010). S1–S6 were diluted by distilled water to 50, 250 and 500 μg/ml, respectively. Two millilitres of 0.1 mM DPPH in methanol was added to 1 ml of the sample solution. The absorbance was measured at 517 nm after 30 min of incubation at 25 °C. Methanol instead of DPPH was used for the blank, whilst distilled water instead of sample was used for the control. The DPPH radical scavenging activity of the sample was calculated by the following equation:

$$\text{DPPH radical scavenging activity } (\%) = \left[1 - \left(A_{\text{sample}} - A_{\text{blank}}\right)/A_{\text{control}}\right] \times 100,$$

where A_{sample}, A_{control} and A_{blank} are the absorbances of sample, control and blank, respectively.

2.6. Statistical analysis

All the tests were conducted in triplicate. The results obtained were subjected to one-way analysis of variance. Duncan's new multiple range test was performed to determine the significant difference between samples within the 95% confidence interval using SPSS 11.5 software (SPSS Inc., Chicago, Illinois, USA).

3. Results and discussion

3.1. Protein contents of S1–S6

The protein contents of S1–S6 were determined to evaluate the extraction efficiency under different conditions. As shown in Table 1, the protein contents of S1 before and after TCA precipitation were 1.57 ± 0.11 and 0.69 ± 0.06 mg/ml, respectively. Ultrasound treatment in combination with enzymatic hydrolysis could improve them to 4.12 ± 0.16 and 2.06 ± 0.09 mg/ml, respectively. The protein contents before and after TCA precipitation were in a decreasing order, S6 = S5 = S4 > S3 > S2 > S1. These results indicated that enzymatic hydrolysis in classical water bath could significantly improve the yield of SSLP and the extraction efficiency was significantly ($p < 0.05$) higher than ultrasound treatment.

Degradation of protein macromolecules into small molecular-weight peptides with higher water solubility was hypothesised to be a mechanism responsible for the high extraction efficiency (Ortiz & Wagner, 2002). TCA, as an protein coagulant, can induce the precipitation of proteins by unfolding the structure (Grimbleby & Ntailianas, 1961; Sivaraman, Kumar, Jayaraman, & Yu, 1997). The peptide chains with low molecular weight cannot be precipitated easily. In this work, all the SSLP hydrolysates showed a significant increase of protein content after TCA precipitation, comparing with native SSLP (Table 1). This result further confirmed the hypothesis. Furthermore, breaking the linkages between proteins and other

Table 1
Protein contents (mg/ml) of SSLP hydrolysates and native SSLP before and after TCA precipitation.

Samples	Before TCA precipitation	After TCA precipitation
S1	1.57 ± 0.11a	0.69 ± 0.06a
S2	4.12 ± 0.16b	2.06 ± 0.09b
S3	5.18 ± 0.16c	2.24 ± 0.05c
S4	6.39 ± 0.32d	2.64 ± 0.09d
S5	6.16 ± 0.28d	2.58 ± 0.10d
S6	6.25 ± 0.25d	2.53 ± 0.05d

components of plant tissues might be another mechanism for the increased dissolution of proteins. Comparing with traditional water bath treatment, ultrasound was shown to have a negative effect on the enzymatic hydrolysis. When 30 min of hydrolysis time was applied, S5 had a protein content of 6.16 ± 0.28 mg/ml, whilst the ultrasound-treated SSLP hydrolysate, S2, was only 4.12 ± 0.16 mg/ml. Though Li, Yoshimoto, Tsukuda, Fukunaga, and Nakao (2004) have suggested the enhancement of ultrasound irradiation on enzymatic hydrolysis of cellulase. Jia et al. (2010) have also indicated that ultrasound treatment during proteolysis can facilitate the hydrolysis of wheat germ protein by Alcalase. However, this enhancement effect was not found for Alcalase in this work. The difference in substrate might be responsible for this behaviour.

3.2. Molecular weight distribution of S1–S6

The molecular weight distribution of S1–S6 were analysed by high performance liquid chromatography (Table 2). S1 had the highest percentage (24.95%) of >10 KDa protein fraction than S2–S6. However, the percentages of its 5–10, 3–5, 1–2 and <1 KDa fractions were lower. S2, the ultrasound-treated SSLP hydrolysates, was observed to have the highest percentage (31.50%) for 5–10 KDa fraction. For SSLP hydrolysates prepared by classical water bath treatment, gradual decreases of >10 and 5–10 KDa fractions and increase of 3–5 KDa fraction were observed. No significant ($p > 0.05$) change was found for 2–3, 1–2 and <1 KDa fractions.

Molecular weight is an important parameter reflecting the hydrolysis of proteins, which further correlates with the bioactivity of protein hydrolysates (Li, Jiang, Zhang, Mu, & Liu, 2008). The native SSLP had a high percentage of >10 KDa fraction, whilst only a low percentage of this fraction was found in SSLP hydrolysates. It indicated that this fraction was an important substrate reacting with Alcalase. Alcalase is a non-specific endoprotease produced from bacterial source. It is usually used for limited hydrolysis of proteins and production of bioactive peptides (Kong, Guo, Hua, Cao, & Zhang, 2008). Chabanon, Chevalot, Framboisier, Chenu, and Marc (2007) have mentioned that a behaviour of "zipper" type mechanism was found for rapeseed protein hydrolysates hydrolysed by Alcalase. With the increase of hydrolysis time, >3 KDa fraction keeps decreasing, 1–3 KDa fraction and especially <1 KDa fraction, stays increasing. However, in this work, no significant increase of <1 KDa fraction was found as the hydrolysis time extended. The protein structure might be responsible for the difference. The range of 3–10 KDa was the main molecular weight interval for all the hydrolysates, which accounted for approximate 75%. The percentage increase of 3–5 KDa was originated from the degradation of 5–10 and >10 KDa fractions. S3–S6 had higher percentages of <3 KDa fraction than S2, which suggested that ultrasound treatment inhibited the formation of peptides with very low molecular weight to some extent.

3.3. Amino acid composition

3.3.1. Free amino acid composition

Though Alcalase is an endonuclease, it still leads to the formation of free amino acids in the protein hydrolysates. As shown in Table 3, the total concentration of free amino acids of S1–S6 were 19.45, 47.57, 57.78, 79.10, 83.04 and 84.56 mg/100 ml, respectively. Antioxidant amino acids include Tyr, Met, His, Lys and Trp (Chen, Muramoto, Yamauchi, & Nokihara, 1996). A gradual increase of antioxidant amino acids was observed from S1 to S6. The free amino acid present the highest concentration in native SSLP was Tyr, following by Val, Phe, Leu, Thr and His. Enzymatic hydrolysis could result in fast increase of all the free amino acids. Ser was observed to have the fastest increase during hydrolysis. Its content in S6 (7.27 mg/100 ml) was 18.6-fold of S1 (0.39 mg/100 ml).

Determination of free amino acid formation can well reflect the hydrolysis process (Aguirre, Garro, & de Giori, 2008). The occurrence of free amino acids are involved in the organoleptic characteristics, nutrition value and bioactivity (Nishiwaki & Hayashi, 2001). In this work, the large amounts of Lys, Leu, Ile, Thr, Val and Phe indicated that SSLP hydrolysates were good source of essential amino acids. From the protein contents of S1–S6, it was obvious that there was no significant difference amongst S4–S6. However, from the results of free and antioxidant amino acids, a slight increase could be found amongst them. This result indicated that when the hydrolysis time was more than 15 min, Alcalase further hydrolysed the dissolved proteins in the slurry, not proteins linking with other component of soy sauce lees tissues.

3.3.2. Total amino acid composition

Table 4 presents the total amino acid compositions of native SSLP and its hydrolysates. The total amino acid content of native SSLP was 255.79 mg/100 ml, which was lower than all the SSLP hydrolysates. This was in agreement with the results of the protein content. Different to the results of free amino acids, Glu (37.04 mg/100 ml) was determined to be the amino acid with the highest concentration in native SSLP, following by Asp (24.27 mg/100 ml), Leu (19.94 mg/100 ml), Lys (18.65 mg/100 ml) and Tyr (16.02 mg/100 ml). Cys was present as the lowest concentration (0.84 mg/100 ml) in native SSLP. By comparing the results of free and total amino acids, it was interesting to find that more than half of Cys remained as free amino acid. For most of amino acids, the ultrasound-treated hydrolysate (S2) presented lower contents than classical water bath treated hydrolysates. The total amino acid content of S2 (301.41 mg/100 ml) was apparently lower than that of S4 (934.34 mg/100 ml), though their hydrolysis time was both set as 30 min. This proved the inhibition effect of ultrasound on Alcalase activity to some extent.

The carboxyl side of Glu, Met, Leu, Tyr, Lys and Gln in the peptide linkages are found to be preferentially cleaved by Alcalase (Adamson & Reynolds, 1996). The high proportion of free to total Cys might be due to construction of peptide bonds between Cys and these preferential amino acids. Glu was measured to be the major amino acid in native SSLP, which made it a good substrate of Alcalase and was possible to be extensively hydrolysed. Zarkadas et al. (2007) have investigated the amino acid composition of various soybean cultivars and suggested that Glu is the major amino acid, followed by Asp. This is not consistent with the results obtained in this work.

Table 2
Molecular weight distribution of SSLP hydrolysates and native SSLP.

Samples	>10 (KDa)	5–10 (KDa)	3–5 (KDa)	2–3 (KDa)	1–2 (KDa)	<1 (KDa)
S1	24.95 ± 0.61a	25.39 ± 0.52a	31.76 ± 0.62a	11.79 ± 0.62a	4.48 ± 0.44a	1.63 ± 0.22a
S2	5.55 ± 0.22b	31.50 ± 0.36d	44.12 ± 0.65b	11.12 ± 0.51a	5.63 ± 0.45b	2.08 ± 0.18b
S3	5.50 ± 0.39b	27.48 ± 0.46c	45.68 ± 0.43cd	13.00 ± 0.71b	5.92 ± 0.27bc	2.42 ± 0.19b
S4	5.36 ± 0.27bc	27.27 ± 0.57c	45.32 ± 0.29c	13.44 ± 0.52b	6.24 ± 0.31bc	2.37 ± 0.31b
S5	4.72 ± 0.21cd	26.39 ± 0.61b	46.14 ± 0.39de	13.96 ± 0.41b	6.47 ± 0.38c	2.32 ± 0.23b
S6	4.34 ± 0.31d	26.08 ± 0.35ab	46.90 ± 0.45e	13.98 ± 0.29b	6.40 ± 0.35c	2.30 ± 0.33b

Table 3
Free amino acid compositions of SSLP hydrolysates and native SSLP (mg/100 ml).

Amino acid	S1	S2	S3	S4	S5	S6
Asp	0.25 ± 0.01	0.53 ± 0.04	0.31 ± 0.01	0.80 ± 0.00	0.88 ± 0.04	0.93 ± 0.05
Glu	0.70 ± 0.03	2.72 ± 0.09	3.38 ± 0.05	5.03 ± 0.02	5.12 ± 0.09	5.20 ± 0.05
Ser	0.39 ± 0.02	1.58 ± 0.03	4.31 ± 0.05	6.47 ± 0.12	7.31 ± 0.10	7.27 ± 0.9
Gly	0.18 ± 0.02	1.71 ± 0.05	1.54 ± 0.06	2.34 ± 0.04	2.51 ± 0.08	2.75 ± 0.09
His	1.20 ± 0.03	4.51 ± 0.10	5.63 ± 0.09	7.70 ± 0.09	7.10 ± 0.07	7.14 ± 0.06
Arg	0.70 ± 0.03	3.05 ± 0.06	3.61 ± 0.07	5.18 ± 0.05	5.97 ± 0.07	6.19 ± 0.08
Thr	1.27 ± 0.05	4.02 ± 0.08	5.18 ± 0.02	7.03 ± 0.14	7.08 ± 0.07	7.20 ± 0.08
Ala	1.12 ± 0.01	3.36 ± 0.05	4.22 ± 0.09	6.14 ± 0.07	6.28 ± 0.05	6.20 ± 0.04
Pro	0.36 ± 0.03	0.87 ± 0.04	0.70 ± 0.01	1.15 ± 0.05	1.14 ± 0.02	1.12 ± 0.02
Tyr	5.84 ± 0.05	7.10 ± 0.09	8.78 ± 0.04	10.03 ± 0.06	10.25 ± 0.09	10.29 ± 0.07
Val	1.84 ± 0.02	4.09 ± 0.08	4.70 ± 0.07	6.21 ± 0.08	6.76 ± 0.04	6.95 ± 0.06
Met	0.22 ± 0.02	0.23 ± 0.04	0.96 ± 0.07	0.92 ± 0.04	1.14 ± 0.05	1.18 ± 0.04
Cys	0.09 ± 0.03	0.44 ± 0.06	0.60 ± 0.06	0.96 ± 0.00	1.28 ± 0.01	1.33 ± 0.05
Ile	0.88 ± 0.03	3.50 ± 0.07	3.76 ± 0.07	5.71 ± 0.1	5.89 ± 0.08	5.91 ± 0.04
Leu	1.38 ± 0.04	4.78 ± 0.08	5.15 ± 0.11	7.45 ± 0.08	7.46 ± 0.08	7.91 ± 0.03
Trp	0.46 ± 0.02	0.67 ± 0.04	0.53 ± 0.02	0.56 ± 0.02	0.69 ± 0.04	0.71 ± 0.04
Phe	1.41 ± 0.05	2.37 ± 0.07	2.86 ± 0.06	2.67 ± 0.03	2.96 ± 0.06	2.99 ± 0.07
Lys	1.17 ± 0.05	2.03 ± 0.04	1.56 ± 0.04	2.75 ± 0.06	3.22 ± 0.05	3.29 ± 0.06
Antioxidant amino acids	8.89	14.54	17.46	21.96	22.40	22.61
Sum	19.45	47.57	57.78	79.10	83.04	84.56

Table 4
Total amino acid compositions of SSLP hydrolysates and native SSLP (mg/100 ml).

Amino acids	S1	S2	S3	S4	S5	S6
Asp	24.27 ± 1.23	64.27 ± 1.96	74.90 ± 2.06	109.68 ± 2.53	107.31 ± 2.31	109.98 ± 2.16
Glu	37.04 ± 1.45	90.52 ± 1.52	101.77 ± 2.51	156.95 ± 2.46	162.20 ± 2.55	162.44 ± 2.62
Ser	13.82 ± 1.10	35.44 ± 1.34	38.36 ± 1.64	55.45 ± 2.15	53.57 ± 2.20	54.67 ± 1.35
Gly	12.75 ± 1.08	29.04 ± 1.09	31.88 ± 1.93	46.58 ± 2.55	44.87 ± 1.65	47.82 ± 1.42
His	8.02 ± 1.19	14.56 ± 1.25	15.15 ± 1.26	22.81 ± 1.09	25.78 ± 1.35	22.98 ± 1.84
Arg	13.56 ± 1.34	31.31 ± 0.98	33.00 ± 2.04	46.73 ± 1.06	49.74 ± 1.32	49.30 ± 1.30
Thr	13.95 ± 1.67	31.20 ± 0.84	33.71 ± 1.44	47.63 ± 1.86	48.36 ± 1.95	48.54 ± 1.29
Ala	14.02 ± 1.42	34.71 ± 1.47	37.68 ± 1.26	54.18 ± 1.34	53.28 ± 1.47	55.49 ± 0.76
Pro	10.30 ± 0.96	24.31 ± 1.58	29.18 ± 1.09	39.10 ± 1.30	40.04 ± 1.26	42.99 ± 1.26
Tyr	16.02 ± 0.86	36.29 ± 1.29	33.11 ± 1.55	45.45 ± 1.09	46.40 ± 1.08	49.22 ± 1.49
Val	14.83 ± 1.52	38.36 ± 1.22	39.49 ± 1.04	55.93 ± 0.95	52.34 ± 1.06	49.51 ± 1.24
Met	3.12 ± 0.80	5.41 ± 0.50	3.85 ± 0.56	3.80 ± 0.56	4.55 ± 0.86	4.70 ± 1.67
Cys	0.84 ± 0.34	1.41 ± 0.51	0.88 ± 0.42	1.28 ± 0.62	1.84 ± 0.66	1.92 ± 1.53
Ile	12.30 ± 1.62	29.44 ± 1.26	31.31 ± 1.32	46.50 ± 1.85	44.89 ± 1.26	42.75 ± 1.82
Leu	19.94 ± 0.67	44.14 ± 1.11	54.62 ± 2.31	75.38 ± 1.85	76.07 ± 2.31	79.00 ± 0.95
Trp	10.79 ± 0.91	30.32 ± 1.62	36.53 ± 1.50	42.53 ± 2.04	44.11 ± 2.02	43.94 ± 1.05
Phe	11.57 ± 1.46	29.84 ± 1.24	29.29 ± 1.09	45.35 ± 2.16	42.38 ± 1.73	40.69 ± 1.28
Lys	18.65 ± 1.75	30.86 ± 1.35	29.83 ± 1.62	39.02 ± 2.22	33.82 ± 1.74	35.82 ± 1.37
Antioxidant amino acids	56.60	117.44	118.47	153.61	154.66	156.66
Sum	255.79	601.41	654.53	934.34	931.55	941.76

3.4. DPPH radical scavenging activity

Fig. 1 presents the DPPH radical scavenging activities of S1–S6 at 50, 250 and 500 μg/ml, respectively. A dose-dependent manner was observed for all the sample tested. When 500 μg/ml was applied, S3–S6 exhibited full scavenging activity against DPPH radicals, whilst 66.0% of scavenging activity was detected for S1. At protein concentration of 250 μg/ml, S2–S6 had significantly ($p < 0.05$) higher DPPH radical scavenging activities than S1, but they were significantly ($p < 0.05$) lower than at 500 μg/ml. However, the native SSLP showed a similar scavenging activity to that at 500 μg/ml. To further investigate the antioxidant potential of S1–S6, 50 μg/ml of protein concentration was used. At this concentration, the DPPH radical scavenging activity was in an increasing order, S1 < S2 < S3 < S4 = S5 = S6.

The mechanism of DPPH radical scavenging assay is based on the reduction of $DPPH^{-}$ solution in the presence of a hydrogen donation antioxidant, inducing the formation of non-radical form DPPH-H (Yang, Zhao, Shi, Yang, & Jiang, 2008). The results in this work indicated that enzymatic hydrolysis could significantly improve the antioxidant potential of SSLP. With the extension of hydrolysis time, protein fractions with large molecular weight were degraded into relatively small molecular weight fractions.

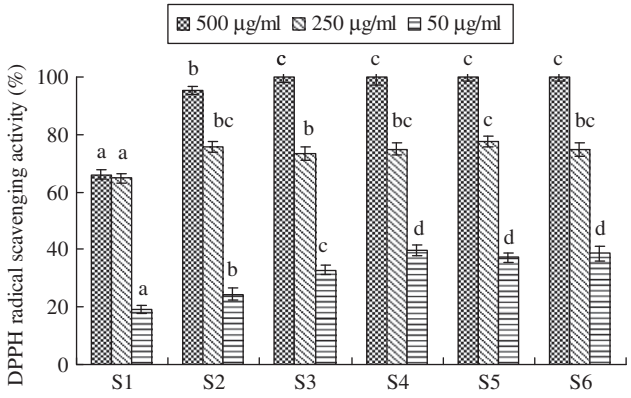

Fig. 1. DPPH radical scavenging activity of SSLP hydrolysates and native SSLP. The columns at the same concentration having the same letters are not significantly ($p > 0.05$) different.

The analyses of molecular weight distribution and amino acid composition confirmed this behaviour. It could be one of the mechanisms leading to the increase of DPPH radical scavenging activity. The antioxidant amino acids contributed much to the antioxidant activity of protein hydrolysates (Murase, Nagao, & Terao, 1993; Rajapakse, Mendis, Jung, Je, & Kim, 2005). The proportion of antioxidant amino acids to total amino acids was in a decreasing order, S1 > S2 > S3 > S4 = S5 = S6. However, the DPPH radical scavenging activity of S1–S6 was in a reverse sequence. It suggested that steric structure and molecular weight of peptides might exert more important role in scavenging DPPH radicals than the content of antioxidant amino acids.

4. Conclusions

It was confirmed in this work that enzymatic hydrolysis could significantly improve the extraction efficiency of SSLP. Ultrasonic treatment showed an inhibition effect on the enzymatic hydrolysis of SSLP to some extent. Tyr was the major free amino acid of S1–S6, whilst Glu was the major one in total amino acid composition. All the hydrolysates showed strong DPPH radical scavenging activities in a dose-dependent manner. However, the mechanism of ultrasound treatment on inhibiting the enzymatic hydrolysis is still not clear, which will be carried out in our future work.

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