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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