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ORIGINAL PAPER

Debittering effect of *Actinomucor elegans* peptidases on soybean protein hydrolysates

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Abstract Effects of the enzymes in *Actinomucor elegans* extract and the enzyme Alcalase 2.4L on debittering the soybean protein hydrolysates were investigated. When the protein was treated only with the latter, a strong bitterness formed; but it decreased if the protein was treated with both the enzymes. The more the enzymes were used, weaker was the bitterness tasted. SDS-PAGE profile and ESI-MS spectrum of the hydrolysates evidenced that the Alcalase could convert the protein into peptides rapidly, while the enzymes in the *A. elegans* extract were able to further degrade some peptides which were difficult or unable to be hydrolyzed by the Alcalase. Further systematic analysis of the peptidases showed that the Alcalase exhibited a significant endopeptidase activity towards NBZ-Phe-pNA substrate ($p < 0.01$), whereas many exopeptidases in the *A. elegans* extract had the carboxypeptidase activity towards *N*-CBZ-Ile-Leu ($p < 0.01$). It is concluded that those exopeptidases presented in the *A. elegans* extract can benefit by decreasing the bitterness of the soybean protein hydroysate. They are also capable of being used with the Alcalase in a single-step enzymatic reaction to prepare the bitterless protein hydrolysate, which may be an efficient application for food industry.

Keywords *Actinomucor elegans* · Exopeptidase · Endopeptidase · Soybean protein hydrolysis · Bitterness

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Introduction

Proteolytic enzyme treatment has been commonly used to improve the chemical, functional, and nutritional properties of food proteins. The most commonly used proteins for producing food grade hydrolysates include casein, whey, and soy proteins. Although the enzymatic treatment can provide many targeted desirable characteristics, it may also introduce some undesirable attributes to the products. For example, bitterness is one of the major undesirable aspects of protein hydrolysates for various applications, particularly for beverages.

Bitterness taste appears to be closely related to the content and sequence of hydrophobic amino acids in peptides. Ney hypothesized that the degree of hydrophobicity was the most important predicator of the peptide bitterness, and thus proposed the Q rule [19, 20] that was defined as the sum of the free energies of transfer of the amino acid side chains from ethanol to water, divided by the number of amino acid residues in the peptide. Ishibashi et al. [10] established a theory of the relationship between the peptide structure and the bitterness, which stated that the bitterness of peptides increased with the increasing hydrophobicity of

the C-terminal residue, whereas a basic amino acid, or a hydrophobic amino acid was located at the N-terminal position [11]. Intense bitterness is also associated with peptides having at least two hydrophobic amino acids at the C-terminal [21]. In addition, peptide bitterness may increase with the number of Leu, Phe, and Tyr residues [9, 12, 17].

Many attempts have been made to decrease bitterness by hydrolyzing the bitter peptides with efficient, cost-effective, and environmentally compatible exopeptidases [19, 20], which can also be used together with some proteases to produce bitterless protein hydrolysates in a single-step enzymatic reaction. The *A. elegans* has been commonly used as a starter for preparation of sufu, a traditional Chinese fermented soybean curd which has been served as an appetizer in China for more than 1,000 years [7]. During the sufu fermentation, the *A. elegans* proteases [12] catalyze the degradation of proteins into small molecular weight peptides and amino acids [4], which contribute some special flavors and textures of the products [27]. Following our previous work that used the proteases from *Mucor piriformis Fischer* and *A. elegans* for preparation of the bitterless soybean peptides, this research aimed to further elucidate the debittering effect of the *A. elegans* peptidases in the hydrolysis system so as to explore their more and better industrial application for making bitterless products.

Materials and methods

Cultures and culture conditions

Actinomucor elegans AS3. 2778 was generously provided by Wang Zhihe sufu company, Beijing, China. The inoculum culture was prepared by inoculating the *A. elegans* spores with an inoculating needle from a slope culture into the tofu cake medium in a 250 mL flask, which was previously sterilized at 121 °C for 15 min. The culture was incubated at 28 °C for 72 h. The spore suspension was prepared by suspending the contents of inoculum culture into 100 mL sterile water. Wheat bran (10 g) with water (12 mL) in a 250 mL flask was autoclaved for 45 min at 121 °C. After the spore suspension (2 mL; containing about 3×10^6 viable propagules/mL) was uniformly dispensed into the wheat bran medium in the flasks, the flask containers were then incubated at 25 °C for 2 days.

Actinomucor elegans extraction

After incubation, the solid mold medium was mixed thoroughly with 100 mL sterile distilled water and left to soak at 4 °C overnight. The fermented extract was then squeezed out through a cheese cloth and centrifuged (RCF: 4,020×*g*; 4 °C; HITACHI CR 22G) to remove the insolubles. The clear supernatant of the extract was then assayed for its protease activity and peptidases activity.

Protease assay

One unit (U) of the protease activity was defined as the amount of enzymes that could liberate 1 μg of tyrosine from the substrate protein under the assay conditions. The substrate casein was dissolved to a final concentration of 2% (W/V) by 20 mmol/L of pH 7.0 NaH_2PO_4–Na_2HPO_4 buffer. A mixture of 1 mL substrate solution and 1 mL extract was incubated at 40 °C for 10 min. After the reaction was terminated by 2 mL trichloroacetic acid (TCA) (0.4 mol/L), the tyrosine content of the clear filtrate was determined by the Lowry method, and calculated from a standard calibration curve. A mixture of 1 mL filtrate, 5 mL 0.4 mol/L Na_2CO_3 solution and 1 mL diluted Folin-phenol reagent was incubated at 40 °C for 20 min. Enzyme blanks were also monitored under the same incubation conditions. Absorbance of the product was monitored at 660 nm. The assays were repeated for two different concentrations of extract.

Aminopeptidase, dipeptidyl aminopeptidase and endopeptidase activities

The following enzymatic methods mentioned by Macedo et al. [16] was slightly modified and used for this research. The aminopeptidase activities were measured by using the following substrates: Gly-p-nitroanilide (Gly-pNA), Arg-pNA, Glu-pNA, Leu-pNA, Lys-pNA, and Val-pNA, all dissolved to a final concentration of 16 mmol L^{-1} in methanol. Dipeptidyl aminopeptidase activity was determined by the following substrates: Gly-Phe-pNA, Gly-Pro-pNA, and Phe-Val-pNA, all dissolved to a final concentration of 4 mM in methanol. Endopeptidase activity was assayed using *N*-Benzoyl-Val-Glu-Ile-Asp-p-Nitroanilide (NBZ-Val-Glu-Ile-Asp-pNA), NBZ-Phe-pNA, and NBZ-Gly-Pro-Arg-pNA, all dissolved to a final concentration of 16.4 mM in methanol. All these chemicals in reagent grade were obtained from Sigma.

The reaction mixture, consisting of 2.8 mL potassium phosphate buffer (0.1 mol l^{-1}, pH 7.0), 0.1 mL substrate solution and 0.1 mL extract, was incubated at 37 °C for 6 h. Absorbance of the ρ-nitroanilide moiety released during the reaction was monitored at 410 nm at irregular time intervals. Enzyme and substrate blanks were also monitored under the same incubation conditions. The assays were repeated by two different concentrations of the extract. The concentration of the released ρ-nitroanilide was calculated from a calibration curve prepared in advance. The activity was considered equal to the slope of the straight line fitted to the concentration of pNA (l μmol) versus time (h), where

the two concentrations of the extract were considered as distinct points in the same plot. The activity was expressed as μmol of substrate hydrolyzed h^{-1} ml^{-1} diluted enzyme solution or ml^{-1} *Actinomucor elegans* extract.

Carboxypeptidase activities

A method mentioned by Macedo et al. [16] was used. The substrates, *N*-Carboben zoxy-Ile-Leu (*N*-CBZ-Ile-Leu) and *N*-CBZ-Gly-Tyr that were obtained from Sigma and dissolved to a final concentration of 2.5 mol L^{-1} in 0.01 mol L^{-1} potassium phosphate buffer (pH 7.0), were used to quantitate the carboxypeptidase activity. The reaction mixture, consisting of 1.8 mL substrate solution and 0.2 mL extract, was incubated at 37 °C for up to 6 h. Aliquots (50 μL) were taken and mixed with the Cd-ninhydrin reagent (2 mL), and then incubated at 84 °C for 5 min [6]. The amino acids released were assayed by measuring the absorbance at 507 nm. The enzyme and substrate blanks were also monitored under the same incubation condition, and two different concentrations of each enzyme sample were repeated in the study. The concentrations of free amino acids in the amino acid residues present in each substrate were calculated from the calibration curves prepared in advance. The activity was expressed as μmol of substrate hydrolyzed h^{-1} mL^{-1} *A. elegans* extract.

Enzymatic hydrolysis

Soybean protein isolated (SPI, containing 90.80% protein content, provided by Shangdong Wonderful Plant Protein Co. Ltd, Shangdong province, China) was used as the substrate. The commercial protease Alcalase 2.4L, that was purchased from Novozymes (Bagsvaerd, Denmark), is a food grade endoprotease from Bacillus licheniformis. Its main enzyme component is the serine protease subtilisin A (EC 3.4.21.62). In this study, 1 mL of Alcalase 2.4L was dissolved by 100 ml distilled water before analysis. This enzyme and the enzyme extract from the *A. elegans* culture were used for the protein hydrolysis. The enzymatic activity was determined by the method mentioned in Sect. 2.3.

The experimental design for the debittering test adopted the following enzyme combinations (see Table 1). Samples A to F were prepared by adding 1,000 U Alcalase diluted solution/g SPI with the *A. elegans* extract/g SPI in 0, 200, 400, 600, 800, and 1,000 protease units.

The SPI was diluted to 10% (w/v) with deioned water. Its pH value was in the range of 6.2–6.3 which was optimal for the hydrolysis. The mixture of enzyme and substrate was incubated at a desirable temperature. During the enzymatic hydrolysis in a period of 12 h, aliquots (100 μL) of the hydrolysates were removed at time intervals of 15, 30 and 60 min and immediately placed into the boiling water for 30 min to denature the enzymes and terminate the hydrolysis reaction. The degree of hydrolysis of the hydrolysates was determined. In addition, the obtained hydrolysates were used for the SDS-PAGE analysis. After removing the sludge by a high speed refrigerated centrifuge (RCF: 4020 × g; 4 °C; HITACHI CR 22G), the soybean protein hydrolysate was lyophilized by a freezing dryer (Alpha 1–4) and subject to the ESI-MS analysis.

Table 1 The experimental design for the enzymatic hydrolysis of soybean protein isolate

Samples	Units of the Alcalase 2.4L diluted solution/g SPI	Units of enzymes in the *A. elegans* extraction/g SPI	Concentration of Soybean protein isolated solution (%)
A	1,000	0	10
B	1,000	200	10
C	1,000	400	10
D	1,000	600	10
E	1,000	800	10
F	1,000	1,000	10

A was treated only by 1,000 U Alcalase diluted solution/g SPI

B was treated by the mixture of 1,000 U Alcalase diluted solution/g SPI and 200 U *A. elegans* extract/g SPI

C was treated by the mixture of 1,000 U Alcalase diluted solution/g SPI and 400 U *A. elegans* extract/g SPI

D was treated by the mixture of 1,000 U Alcalase diluted solution/g SPI and 600 U *A. elegans* extract /g SPI

E was treated by the mixture of 1,000 U Alcalase diluted solution/g SPI and 800U *A. elegans* extract/g SPI

F was treated only by 1000U *A. elegans* extract /g SPI

Degree of hydrolysis

The degree of hydrolysis is defined as the percentage of free amino groups cleaved from protein, which is calculated from the ratio of α-amino nitrogen and total nitrogen. The α-amino nitrogen was determined by a modified formal titration method (U.S.P. [26]). A soybean protein hydrolysate sample in a volume of 5 mL was added with 60 mL distilled water, and then it was adjusted to pH 8.2 using 0.1000 mol/L NaOH solution. After 20 mL of previously neutralized 38%(v/v) formaldehyde solution was added, the mixture was titrated by the 0.1000 mol/L NaOH solution to the end point at pH 9.2, which was standardized against potassium hydrogen phthalate. Total nitrogen was determined by the Kjeldahl method [2].

Electrophoresis of hydrolysate

SDS-polyacrylamide gel electrophoresis (SDS-PAGE) (15% acrylamide) was performed according to the method of Laemmli [14]. Standard proteins used as molecular

weight markers (low range) were purchased from Shanghai Bio-Rad (in China), which consisted of rabbit phosphorylase (97,400 Da), bovine serum albumin (66,200 Da), rabbit actin (43,000 Da), bovin carbonic anhydrase (31,000 Da), trypsin inhibitor (20,100 Da) and hen egg white lysozyme (14,400 Da).

Sensory evaluation

Sensory evaluation for bitter taste of the soy protein hydrolysate was conducted by a panel consisting of 10 female and 9 male between the ages of 22 and 40. The panel members were trained for a period of 1 month, four times a week, with quinine standards. The standard solutions were presented in concentrations of 1.0×10^{-4}, 5×10^{-5}, 2.5×10^{-5}, 1.0×10^{-5} and 5×10^{-6} g/mL that were scored 10, 5, 2.5, 1 and 0.5, respectively.

The standard condition for sensory evaluation was maintained at room temperature for soy protein hydrolysate at the concentration of 2% (g/ml, N × 6.25) at pH 6.5. Each sample in amount of 30 mL was served in a 100 mL brown glass jar, and identified by a four-digit code.

Electrospray ionization mass spectroscopy (ESI-MS) analysis

Mass spectrum of the hydrolysates was analyzed by the ESI-MS (ESQUIRE-LC, Bruker) under the positive mode and the MS/MS method. Fragmentation amplitude was set at 1.00 V. Start amplitude and end amplitude were 30.0 and 200.0%, respectively. Nebulizer pressure was 50 psi. Flow rate of the drying gas (N_2) was set at 10.0 L/min. Drying gas (N_2) temperature was 350 °C.

Statistics

Statistic evaluation was conducted by the Microcal Origin V.7.0. Data were expressed as mean ± standard deviation of the samples.

Results and discussion

Debittering effect of the *A. elegans* extract on soybean protein hydrolysate

Table 2 summarizes the debittering effect of the *A. elegans* extract on the soybean protein hydrolysate. The bitterness score is significantly different between the samples **A** (treated by Alcalase) and **F** (treated by the *A.elegans* extract). The sample **A** presented the highest bitterness score (4.0), whereas the sample **F** gave the least value (0.5) although they were treated with the same enzymatic unit (1,000 U/g SPI). The bitterness of the hydrolysate decreased when the *A. elegans* extract and the Alcalase were used together at the same time. The more the *A. elegans* extract was added, the weaker the bitterness was tasted (**B**, **C**, **D**, **E**, **F**).

Table 2 Effect of enzymes in the *A. elegans* extract on soybean protein hydrolysis

Samples	DH (%)	WS N/T N (%)	BV
A	11.4	35	4
B	24.0	54	3
C	28.7	60	2
D	34.1	64	2
E	36.2	70	0.5
F	28.5	50	0.5

DH Degree of hydrolysis, *WSN/TN* water soluble nitrogen/total nitrogen, *BV* bitterness value

SDS-PAGE profile of the soybean protein hydrolysate

After hydrolysis for 15, 30 and 60 min, the hydrolysates were compared by SDS-PAGE analysis (Fig. 1). Peptide profile of the sample **A** (treated by the Alcalase) displayed an extensive degradation. In contrast, that of F (treated by the *A.elegans* extract) nearly had no change. Since the Alcalase is an endoprotease with a broad specificity and has a tendency of hydrolysis at hydrophobic amino acid residues, it usually leaves a nonpolar amino acid residue at the C-terminus of the peptides. Such kinds of peptides usually have a relatively high bitterness [1]. On the contrary, the *A. elegans* extract acts as an exopeptidase that specifically liberates the terminal amino acid and results in high degree of hydrolysis and low bitterness. Adler-Nissen [1] had also mentioned that fungal proteases often produced less bitterness than other proteases. It was attributed to the effect of the peptidase activity present in most fungal protease preparation.

ESI-MS spectra of the soybean protein hydrolysate

Figure 2 shows the ESI-MS spectra of the soy protein hydrolysates. The ESI-MS spectrum of the sample **A** treated by the Alcalase shows ion 1305 (mass-to-charge, m/z) as the dominant peak. The ion 1305 peak decreased markedly in the samples **B** and **C,** and eventually disappeared in the sample **D** with the addition of the *A. elegans* extract. This indicates that the *A. elegans* extract was able to degrade this peptide completely. Other ion peaks at m/z 949, 975, 1148, 1227, 1385, and 1433 in the sample **E** also disappeared after the treatment by the *A. elegans* extract. This result indicates that the presence of some peptidase in

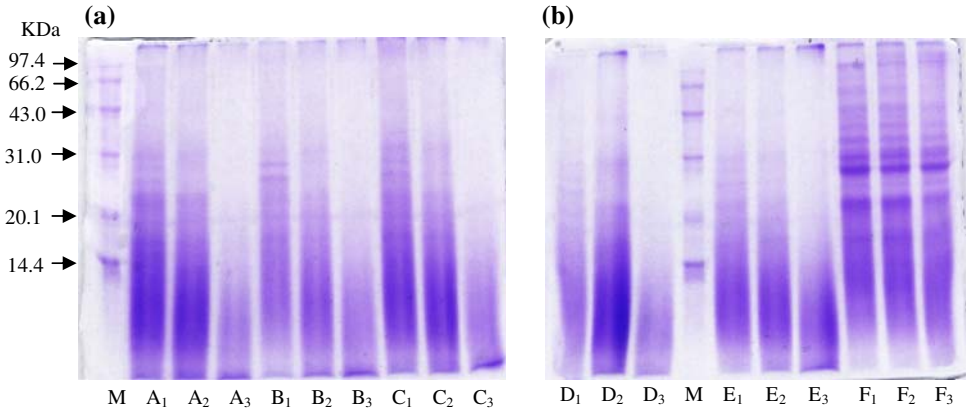

Fig. 1 The SDS-PAGE profiles of the soybean protein hydrolysates. $\mathbf{A}_1$, $\mathbf{A}_2$, $\mathbf{A}_3$: SPI treated by the Alcalase (1,000 μg^{-1} substrate) for 15, 30 and 60 min; $\mathbf{B}_1$, $\mathbf{B}_2$, $\mathbf{B}_3$: SPI treated by the mixture of the Alcalase (1,000 U g^{-1} substrate) and *A .elegans* extract (200 U g^{-1} substrate) for 15, 30 and 60 min; $\mathbf{C}_1$, $\mathbf{C}_2$, $\mathbf{C}_3$: SPI treated by the mixture of Alcalase (1,000 U g^{-1} substrate) and *A. elegans* extract (400 U g^{-1} substrate) for 15, 30 and 60 min; $\mathbf{D}_1$, $\mathbf{D}_2$, $\mathbf{D}_3$: SPI treated by the mixture of Alcalase (1,000 U g^{-1} substrate) and *A. elegans* extract (600 U g^{-1} substrate) for 15, 30 and 60 min; $\mathbf{E}_1$, $\mathbf{E}_2$, $\mathbf{E}_3$: SPI treated by the mixture of Alcalase (1,000 U g^{-1} substrate) and *A. elegans* extract (800 U g^{-1} substrate) for 15, 30 and 60 min; $\mathbf{F}_1$, $\mathbf{F}_2$, $\mathbf{F}_3$: SPI treated by the *A. elegans* extract only (1,000 U g^{-1} substrate) for 15, 30 and 60 min; *M* Marker, standard molecular mass markers of rabbit phosphorylase (97.4 KDa), bovine serum albumin (66.2 KDa), rabbit actin (43.0 KDa), bovin carbonic anhydrase (31.0 KDa), trypsin inhibitor (20.1 KDa) and hen egg white lysozyme (14.4 KDa)

the *A. elegans* extract might have exerted a further degradation of the soybean protein hydrolysates after the Alcalase treatment. However, some larger ions such as 1794, 1666, and 1621 peaks seem exceptional.

Figure 2**F** shows the ESI-MS spectrum of the sample **F** that was only treated by the *A. elegans* extract. The spectrum of **F** had ions at m/z 1581, 1909, and 2284 as the dominant peaks but did not show the peaks of 1227, 1305, and 1666. This result indicates that some endopeptidases might also exist in the *A. elegans* extract that led to the formation of those above peptides.

From Table 2 and Fig. 2, it is also implied that parts of the peptides, of which their ion peaks are at m/z 1305, 949, 975, 1148, 1227, 1385, and 1433 respectively, may be related to the bitterness, but further research on the taste of these peptides is needed.

Peptidase activities of the *A. elegans* extract

Table 3 displays the amino peptidase activity, dipeptidyl amino peptidase activity, endopeptidase activity, and carboxypeptidase activity presented by the *A. elegans* extract and the Alcalase. The latter did not have the amino peptidase activity, but exhibited a significant endopeptidase activity towards the Phe site ($p<0.01$). On the contrary, the *A. elegans* extract showed the amino peptidase activity towards the substrates of Glu-pNA, Gly-pNA, Arg-pNA, Leu-pNA, Lys-pNA, and Val-pNA; the dipeptidase activity towards the substrates of Gly-Phe-pNA, Gly-Pro-pNA, and Phe-Val-pNA; and the carboxypeptidase activity towards the *N*-CBZ-Ile-Leu. Shinoda et al. [21] pointed out that the bitterness of peptides increased with the hydrophobicity of the C-terminal residue when a basic amino acid or a hydrophobic amino acid was located at the N-terminal position. Since the enzymes in the *A. elegans* extract exerted significant exopeptidase activities towards the hydrolysates (substrates) produced by the Alcalase treatment, the hydrophobic amino acids at the C-terminals were cleaved and removed, which resulted in a significant decrease in the bitterness of soybean protein hydrolysate after the *A. elegans* treatment. Such kind of complementary activities of both the enzymes were evidenced by and in agreement with the results from the SDS-PAGE, ESI-MS, and DH analyses.

Table 3 also shows that the glycine aminopeptidase exists in the *A. elegans* extract. Firstly reported by Ito et al. [13], this peptidase from the *A. elegans* could highly specifically hydrolyze Gly-X (amino acid, peptide, or acrylamide) bonds. According to the research of Matoba et al. [17], peptides could become more bitter than the original one when their amino and carboxyl groups were blocked, and new peptide bonds were formed. Although leucine, proline, tyrosine, and valine were slightly bitter or tasteless at the tasted concentration, peptides coupled with glycine in their amino and carboxyl groups could become very bitter. Besides, Ma et al. [18] found that the glycine amino peptidase was able to improve the protease activity considerably in the process of protein hydrolyzing.

Table 3 shows that the carboxypeptidase activity towards N-CBZ-Ile-Leu is significantly high in the *A. elegans* extract ($p<0.01$). Fungal carboxypeptidases, such as *Aspergillus* acid carboxypeptidase [22–25] and *Mucor racemosus* carboxypeptidase [5], are usually a serine carboxypeptidase with a broad specificity. Arai et al. [3] reported the bitterness of peptides from soybean protein hydrolysates

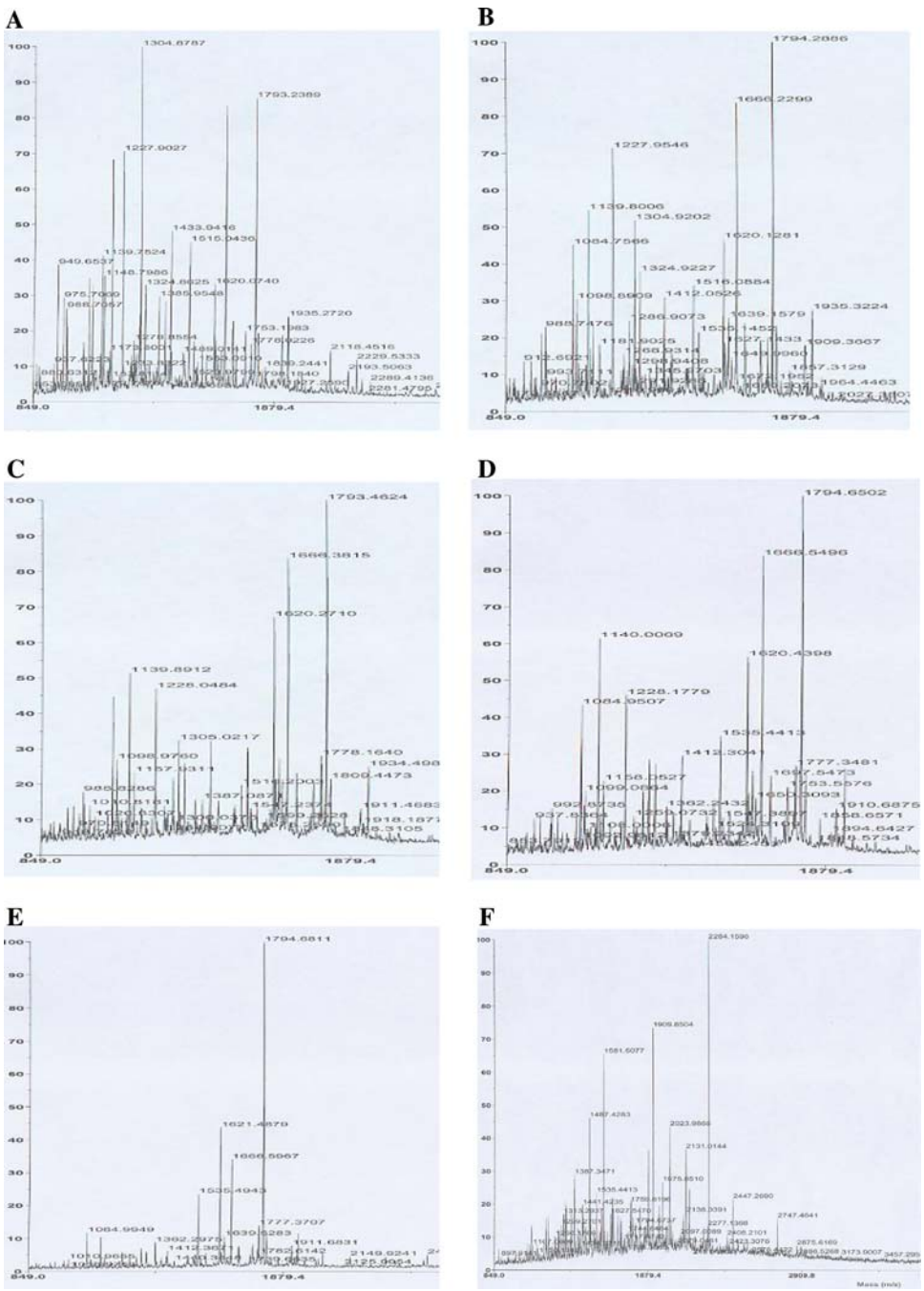

Fig. 2 ESI-MS spectra of soybean protein hydrolysates. (A) sample **A**, soybean protein hydrolysate treated only by the Alcalase (1000 U g^{-1} SPI); (B) sample **B**, soybean protein hydrolysate treated by the mixture of Alcalase (1,000 U g^{-1} SPI) and *A. elegans* extract (200 U g^{-1} SPI); (C) sample **C**, soybean protein hydrolysate treated by the mixture of Alcalase (1,000 U g^{-1} SPI) and *A. elegans* extract (400 U g^{-1} SPI); (D) sample **D**, soybean protein hydrolysate treated by the mixture of Alcalase (1,000 U g^{-1} SPI) and *A. elegans* extract (600 U g^{-1} SPI); (E) sample **E**, soybean protein hydrolysate treated by the mixture of Alcalase (1,000 U g^{-1} SPI) and *A. elegans* extract (800 U g^{-1} SPI); (F) sample **F**, soybean protein hydrolysate treated only by *A. elegans* extract (1,000 U g^{-1} SPI)

decreased after the treatment of *Aspergillus* acid carboxypeptidase. Recently, Liu et al. [15] found that *Monascus pilosus* carboxypeptidase (MpiCP-1) could suppress and reverse the development of the bitterness taste that resulted from the pepsin hydrolysis of soybean protein by releasing mainly the hydrophobic amino acids from the C-terminal of the bitter components. Regardless of the new findings of the different peptidase activities of the *A. elegans* exact, further investigation for purification and specificity determination of the *A. elegans* carboxypeptidase is needed and conducted in our labs.

Conclusions

The bitterness of the soybean protein hydrolysates produced by the Alcalase 2.4L decreased when the hydrolysates were treated together with the *A. elegans* extract. The more the extract was used, weaker was the bitterness formed. The Alcalase is a typical endopeptidase which can convert the protein into peptides rapidly, whereas the *A. elegans* extract is able to further degrade some peptides which are difficult or unable to be hydrolyzed by the Alcalase itself. The Alcalase exhibited a significant endo-

Table 3 Peptidase activities (average ± standard error) of the Alcalase and the *A. elegans* extract

Substrates	Alcalase diluted solution	*Actinomucor elegans* extract
Gly-pNA[a]	ND	0.2750 ± 0.0071
Glu-pNA	ND	0.0380 ± 0.0156
Leu-pNA	ND	0.1450 ± 0.0354
Lys-pNA	ND	0.1260 ± 0.0014
Arg-pNA	ND	0.1075 ± 0.0035
Val-pNA	ND	0.1065 ± 0.0021
Gly-Phe-pNA	ND	0.1785 ± 0.0304
Gly-Pro-pNA	2.2000 ± 0.1414	0.3335 ± 0.0050
Phe-Val-pNA	0.1100 ± 0.0141	0.0675 ± 0.0177
NBZ-Val-Glu-Ile-Asp-pNA[b]	3.1000 ± 0.1414	0.0675 ± 0.0177
NBZ-Phe-pNA	23.900 ± 0.1414	0.0285 ± 0.0021
NBZ-Gly-Pro-Arg-pNA	3.3500 ± 0.2121	0.0570 ± 0.0028
N -CBZ-Ile-Leu[c]	3.5355 ± 0.0205	5.7405 ± 0.2949
N -CBZ -Gly-Tyr	0.2915 ± 0.0021	ND

Peptidase activities expressed as μmol of substrate h^{-1} mL^{-1} of Alcalase diluted solution or the *A . elegans* extract

ND not detected

[a] pNA = p-nitroanilide p-nitroanilide

[b] NBZ = *N*-Benzoyl

[c] *N*-CBZ = *N*-Carboben zoxy

peptidase activity towards the NBZ-Phe-pNA substrate ($p < 0.01$), whereas the *A. elegans* extract contained some exopeptidases benefiting to the debittering of the soybean protein hydroysate. This one step enzymatic reaction by using the Alcalase and the exopeptidases in the *A. elegans* extract may help in simplifying the industrial process for preparing bitterless protein hydrolysates.

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