



Analytical Methods

Comparison of hydrolysis characteristics on defatted peanut meal proteins between a protease extract from *Aspergillus oryzae* and commercial proteases

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ABSTRACT

A crude protease extract (CPE) was prepared from *Aspergillus oryzae* HN 3.042 in this work. Three commercial proteases (Alcalase 2.4L, Protamex and papain) and CPE were employed to hydrolyse defatted peanut meal (DPM). CPE was found to be the most effective protease with protein recovery of 80.6%. Moreover, CPE produced a higher degree of hydrolysis (DH, 43.4%) than the tested commercial proteases. The test of molecular weight distribution indicated that DPM proteins were mainly consisted of >10 KDa fraction (86.6%), whereas 3–6 KDa fraction was observed to be the main fraction of all the hydrolysates. CPE hydrolysate possessed a higher nutritional quality than DPM and other hydrolysates on the basis of FAO/WHO (1991) reference pattern. The sensory taste evaluation showed that CPE hydrolysate had better taste than other hydrolysates.

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1. Introduction

Peanut is an important oilseed crop over the world. In China, the annual yield of peanut in 2007 was more than 13 million tons, which ranks the first of the world (Wu, Wang, Ma, & Ren, 2009). Defatted peanut meal (DPM) is the main byproduct during peanut oil production, which is rich in proteins with large amount of essential amino acids (Basha & Pancholy, 1982). However, DPM is easily to be deteriorated during defatting process, which results in poor protein solubility, dark colour, unpleasant flavour and low nutrition value (Robin & Chiou, 1990). At present, DPM is mainly used as animal feed and fertiliser. However, the application in food industry is still limited.

The functional properties and nutrition value of proteins can be improved by enzymatic hydrolysis (Neucere, Conkerton, & Booth, 1972; Yu, Ahmedna, & Goktepe, 2007), which can generate polypeptides and free amino acids (Guerard, Dufosse, Broise, & Binet, 2001; Quist, Phillips, & Saalia, 2009). Polypeptides with low molecular weight can be absorbed by the intestine directly and have low allergenic effects. The biological functions of polypeptides have been reported, including antihypertension, opioid agonists or antagonists, immunomodulatory, antithrombotic, antioxidant, anti-cancer, and antimicrobial activities in addition to nutrient utilisation (Clare & Swaisgood, 2000; Elias, Kellerby, & Decker, 2008). Therefore, it is an interesting attempt to utilise DPM

proteins to prepare polypeptides. Research has been done on the hydrolysis of DPM proteins by Alcalase, Protamex and papain (Govindaraju & Srinivas, 2004). However, the hydrolysis efficiencies of these commercial proteases are relatively low (Vioque et al., 1999). Moreover, the hydrolysates have bitter taste, which is not desirable for food use.

Aspergillus oryzae has been used during the production of traditional soy sauce in Asia, which can produce proteases for protein hydrolysis (Blinkovsky, Byun, Brown, Golightly, & Klotz, 2000). It is reported that *A. oryzae* has the largest expansion of hydrolytic genes (Vishwanatha, Rao, & Singh, 2009). Agrawal, Patidar, Banerjee, and Patil (2004) have reported that fungi-derived alkaline proteases exhibit high protease activity and have the potential as commercial application to soy protein hydrolysis. So far, little information is available concerning the utilisation of *A. oryzae* on peanut protein hydrolysis.

The objective of this work was to utilise the crude protease extract of *A. oryzae* HN 3.042 (CPE) to hydrolyse DPM. Commercial proteases such as Alcalase, Protamex and papain were used for comparison. The nutritional value and sensory taste of the hydrolysates were evaluated.

2. Materials and methods

2.1. Materials and chemicals

Defatted peanut meal was purchased from Shandong Luhua Group Co., Ltd. (Shandong, China). It contained 49.1% protein,

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31.5% carbohydrate, 6.3% moisture, and 4.5% ash. Alcalase 2.4L and Protamex were purchased from Novozymes (Beijing, China). Papain was obtained from Baiao Biochemistry Co. (Jiangmen, China). All the other chemicals and solvents were of analytical grade.

2.2. Preparation of crude protease extract

Wheat bran (20 g), defatted peanut flour (8 g), calcium chloride (0.028 g) and deionised water (28 g) were mixed and sterilised in a conical flask at 121 °C for 30 min. Then the mixture was cooled to 38 °C and inoculated with spores of *A. oryzae* HN 3.042 (14.93×10^9 spores/g of protein). The obtained mixture was then cultured at 30 °C for 68 h. At the end of fermentation, 100 mL of 0.2 M phosphate buffer (pH 7.0) was added and vigorously mixed for 1 h. The mixture was then centrifuged at $10,000 \times g$ for 30 min at 4 °C (GL-21 M refrigerated centrifuge Xiangyi Instrument Co. Ltd., Changsha, China). The obtained supernatant (CPE) was then lyophilised (R2L-100KPS, Kyowa Vacuum Engineering, Tokyo, Japan) for further use.

2.3. Protease activity assay

The proteolytic activities of CPE, Alcalase, Protamex and papain were determined as follows: casein (2%, w/v) prepared in 0.2 M buffer at desirable pH (sodium phosphate for pH 6 and 7; Tris-HCl for pH 8). A mixture of 0.25 mL of casein solution and 0.25 mL of enzyme extract (1:200 diluted for CPE and 1:1000 for commercial proteases with the appropriate buffer) was incubated at 30 °C for 10 min. The reaction was terminated by adding 0.5 mL of 0.4 M trichloroacetic acid. The mixture was then centrifuged at $10,000 \times g$ for 15 min at 4 °C. The supernatant (0.25 mL) was added to 1.25 mL of 0.4 M sodium bicarbonate followed by 0.25 mL of 0.4 M Folin-Ciocalteu phenol Reagent (1:3 diluted in water). The reaction was allowed to stand for 30 min at 30 °C. Absorbance was read against a blank at 660 nm. Samples were analysed in triplicates. One unit of protease (U) was defined as the amount of enzyme which yields the colour equivalent to 1 μ M of tyrosine in 1 mL of reaction solution per minute at 30 °C.

2.4. Preparation of defatted peanut meal hydrolysates (DPMH)

Fifty grams of DPM were added into 50 mL of deionised water and heated at 121 °C for 15 min using an autoclave (Shanghai Shenan Instrument Co., Ltd., Shanghai, China), then mixed with 400 mL of deionised water and homogenised at a speed of 10,000 rpm for 1 min using an Ultra Turrax homogeniser (Beijing Jingke Huarui Instrument Co., Ltd., Beijing, China). The homogenate was subjected to enzymatic hydrolysis by CPE, Alcalase 2.4L, Protamex and papain, respectively. The pH of suspension was adjusted with 1 M NaOH. The detailed hydrolysis procedures are described in Table 1. At the end of hydrolysis, the enzyme was inactivated by heating in a boiling water bath for 15 min. The hydrolysates were centrifuged in a GL-21M refrigerated centrifuge (Xiangyi Instrument Co. Ltd., Changsha, China) at $5000 \times g$ for 20 min at 20 °C and the supernatants were collected, lyophilised (R2L-100KPS, Kyowa Vacuum Engineering, Tokyo, Japan) and stored in a desiccator for further analysis.

Table 1
Hydrolysis conditions of different proteases on DPM.

Sample	pH	Protease activity (U)	Time (h)	Temperature (°C)	E/S ratio (w/w)
CPE	7	15,478	24	55	4/1000
Alcalase 2.4 L	8	105,869	24	60	1/1000
Protamex	6	82,480	24	50	1/1000
Papain	7	63,000	24	50	1.5/1000

2.5. Determination of protein recovery and degree of hydrolysis (DH) of DPMH

DH is defined as the percentage of free amino groups cleaved from proteins, which was calculated from the ratio of α -amino nitrogen to total nitrogen. The amino nitrogen content was determined by formaldehyde titration method (Nilsang, Lertsiri, Supphantharika, & Assavanig, 2005). The total nitrogen content was determined by Kjeldahl method (the conversion factor is 5.46 for peanut protein) (Miller & Young, 1977). Protein recovery was calculated as the ratio of protein amount (Nitrogen percentage $\times$ 5.46) present in the hydrolysates to that in the reaction mixture (Miller & Young, 1977).

2.6. Determination of the molecular weight distribution of peptides

Molecular weight distributions of peptides in the supernatants from the DPM and its hydrolysates were determined by gel permeation chromatography. A protein purification chromatography (Amersham plc, Buckinghamshire, United Kingdom) with a Superdex Peptide 10/300 GL column was used for the analysis. The mobile phase (isocratic elution) was 0.02 M sodium phosphate buffer containing 0.25 M NaCl (pH 7.2), at a flow rate of 0.5 mL/min. Absorbance was monitored in the range 200–350 nm, the value at 214 nm were recorded. The water-soluble peptide fraction was filtered through a micropore film (0.22 μ m of pore size). Six protein standards, Globin III (2512 Da), Globin II (6214 Da), Globin I (8519 Da), Globin I + III (10,700 Da), Globin I + II (14,404 Da) and Globin (16,949 Da) were taken to make reference curve. The molecular weight of peptides was calculated by the elution volume. UNICORN 5.0 software (Amersham Biosciences Co., Piscataway, NJ, USA) was used to analyse the chromatographic data.

2.7. Amino acid analysis

The amino acid profiles of DPM and DPMHs were determined according to the method of Bidlingmeyer, Cohen, Tarvin, and Frost (1987) with a slight modification. Amino acid composition was determined by high performance liquid chromatography equipped with a PICO.TAG column (Waters, Milford, MA, USA). Total amino acid residues were determined after hydrolysis at 110 °C for 24 h with 6 M hydrochloric acid prior to the derivatization with phenyl isothiocyanate. The following amino acids were used as external standards, including L-alanine, L-arginine, L-aspartic acid, L-cystine, L-glutamic acid, glycine, L-histidine, L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-proline, L-serine, L-threonine, L-tyrosine, L-valine and ammonium chloride (Sigma Co., St. Louis, MO, USA). These standards were used at equal concentration except for ammonium chloride. Alkaline hydrolysis was also done for determination of Trp level (Sigma Co.).

The contents of different amino acids were expressed as mg/100 mg protein and compared with the FAO/WHO (1991) reference pattern. The score of essential amino acids (EAAs) was calculated by the recommendation of FAO/WHO as shown below:

Essential amino acids score

$$= \frac{\text{mg of EAA in 100 mg of test protein}}{\text{mg of EAA in 100 mg of recommendation protein}}$$

2.8. Factor analysis

Factor analysis is often used in data reduction to identify a small number of factors that explain most of the variance, which is observed in a much larger number of manifest variables (Riitters

et al., 1995). In this work, Factor analysis used the amino acid composition to identify a small number of factors (significant variables) and to establish the classification of DPM protein and DPMHs.

Because within group correlations of the 18 metrics were large and between-group correlations were small, factor analysis was proceeded by principal component analysis and varimax rotation. The principal component analysis was used to obtain the initial factor solution and to reduce the dimensionality of a data set, which can use fewer factors to explain more variation within the data. Varimax rotation method can minimise the number of variables that had high loadings on each factor. This method simplified the interpretation of factors.

Representative maps were then chosen to illustrate the results of the factor analysis. These choices were based on the factor scores for each map. For a given map and factor, a factor score was calculated as the weight sum of all the 18 metrics, where the weights were the loadings of that factor. In geometric terms, the scores were the relative positions of different maps along the axis corresponding to a given factor.

2.9. Sensory evaluation

Descriptive analysis was performed to determine the differences of sensory flavour characteristics between four DPMHs. Analysis was carried out with a panel of 10 flavorists (five men and five women, aged from 25 to 35) from Guangdong H-BIO Biotechnology Co. Ltd (Guangzhou, China). The assessors were asked to rank four attributes (bitter, umami, salty and full-bodied taste) on a eleven-point linear scale from 0 to 10. Intensities of the four attributes evaluated for CPE were selected as standards and ranked 10. Sensory evaluation was performed in a sensory panel room at 23 ± 2 °C. Hydrolysates (1%, v/v) of CPE, Alcalase, Protamex and papain were chosen for sensory evaluation.

Four sensory attributes of the DPMHs prepared by commercial proteases were evaluated. The average values of each attribute obtained from different assessors were plotted in a spider web diagram. Significant differences between four hydrolysates were obtained by analysis of variance (ANOVA).

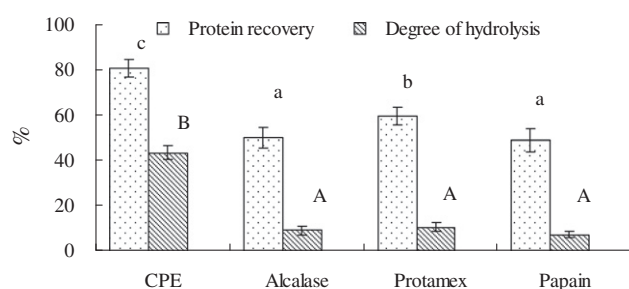


Fig. 1. Protein recovery and DH of DPM hydrolysed by CPE and other commercial proteases. The values in a column followed by different letters are significantly different ($p < 0.05$).

Table 2
The molecular weight distribution of peptides from DPMP (DPM proteins) and DPMHs*.

Molecular-weight range (KDa)	DPMP (%)	Hydrolysates (%)			
		CPE	Alcalase	Protamex	Papain
≥10	86.6 ± 1.40 ^b	0.180 ± 0.08 ^a	0.540 ± 0.15 ^a	0.100 ± 0.07 ^a	0.420 ± 0.12 ^a
6–10	0.990 ± 0.21 ^a	2.99 ± 0.27 ^b	9.51 ± 0.73 ^c	10.7 ± 1.24 ^c	13.2 ± 1.17 ^d
3–6	4.89 ± 1.02 ^a	44.6 ± 2.51 ^b	72.4 ± 3.87 ^c	76.5 ± 2.93 ^c	74.8 ± 2.56 ^c
1–3	6.27 ± 0.40 ^a	35.6 ± 2.51 ^{ab}	16.7 ± 3.16 ^{bc}	13.7 ± 3.04 ^c	9.76 ± 1.60 ^d
<1	1.43 ± 0.24 ^a	15.9 ± 2.16 ^b	0.670 ± 0.20 ^a	0.370 ± 0.11 ^a	0.920 ± 0.13 ^a

* The values in the same row followed by different letters are significantly different ($p < 0.05$).

2.10. Statistical analysis

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

3. Results and discussion

3.1. Protein recovery and DH

Protein recoveries of DPMHs treated by various proteases are shown in Fig. 1. It showed that the protein recovery of the CPE hydrolysate (80.6%) was significantly ($p < 0.05$) higher than other protease hydrolysates. The protein recovery of Protamex hydrolysate was the next (59.4%), following by Alcalase (49.8%) and papain hydrolysates (48.7%).

DH was used as an indicator for the cleavage of peptide linkages. As shown in Fig. 1, the DH of CPE hydrolysate was 43.4%, which was also significantly higher than other proteases ($p < 0.05$). No significant difference was found among the other three proteases ($p > 0.05$).

To explain the high hydrolysis efficiency of CPE, the proteolytic activities were analysed (Table 1). CPE exhibited the lowest proteolytic activity compared with commercial proteases. One of the possible explanations for the inconsistent results is that the analysis of proteolytic activity was based on casein, and CPE might have less affinity to casein than commercial proteases. Additionally, there could be other mechanisms responsible for the high hydrolysis efficiency of CPE on DPM. DPM proteins used as nitrogen source might induce fungi to secrete proteases which could specifically hydrolyse DPM proteins during fermentation. Furthermore, DPM had a large amount of cellulose (Hoffpauir, 1953). CPE might contain cellulolytic enzyme that could decrease the viscosity of the reaction mixture and enhance the protein dissolution, and then accelerate the hydrolysis (Ansharullah & Chesterman, 1997; Guan & Yao, 2008). Ferea, Botstein, Brown, and Rosenzweig (1999) have reported that the fermented mix can drive the organism to synthesise a number of hydrolytic enzymes and transport-related gene families for efficiently utilising external nutrient sources. Therefore, it is reasonable that the endogenous proteases can well hydrolyse the proteins used in the culture medium. Similar DH results has been reported for giant sea bass hydrolysed by the protease extracted from ferment product (García-Gómez, Huerta-Ochoa, Loera-Corral, & Prado-Barragán, 2009).

3.2. The molecular weight distribution of peptides analysis

The molecular weight distribution of peptides was analysed by gel permeation chromatography and the results are shown in Table 2. DPM were dominated by the fractions with molecular weight larger than 10 KDa (86.6%), while all the hydrolysates were rich

in fractions with smaller molecular weight (3–6 kDa). It indicated DPM was greatly degraded by proteases. However, there was difference between CPE hydrolysate and commercial protease hydrolysates. For CPE hydrolysate, the percentages for 3–6 kDa and 1–3 kDa fractions were 44.6% and 35.6%, respectively, while the commercial protease hydrolysates were mainly composed of 3–6 kDa fraction (Alcalase, 72.4%; Protamex, 76.5%; papain, 74.8%). Especially, the content of small peptides (<1 kDa) in CPE hydrolysate was much higher than that in commercial proteases hydrolysates.

This result indicated that CPE was much more effective for producing smaller peptides than commercial proteases. It was hypothesised that CPE was a mixture of endo- and exo-proteases. During the initial hydrolysis, the proteins were degraded into high molecular weight peptides by endoproteases, then the exoproteases treated sequentially to dissociate them into smaller fragments (Hrčková, Rusňáková, & Zemanovič, 2002; Quist et al., 2009). Similar result has been reported by Guerard, Guimas, and Binet (2002), who has found that the use of Umamizyme from *A. oryzae* can yield a high quantity of small peptides below 0.5 kDa.

3.3. Factor analysis

After factoring the correlation matrix by the principal components method, Table 3 shows the contribution of each factor to the total data set. The eigenvalue from factor analysis indicated that the first factor accounted for 46.1% of the standardised variance, while the second accounted for 29.6% and the third only 19.9%. Thus, the first three factors encoded 95.6% (more than 85%) of the eighteen-variable dataset. Thus, these three factors were selected to represent the initiatory information and its reliability was 95.6%.

The loadings of each metric on each of the first three factors after orthogonal rotation are shown by the factor pattern (Table 3). The dimensions were interpreted by examining the factor pattern. The first axis was nutritional quality because it was highly correlated with measures of average (His, Val and Leu) and large EAAs (Thr, Met, Ile, Trp, Phe, Ser and Lys). The second axis was highly

correlated with measures of umami amino acids (Glu, Asp and Tyr) and sulphur-containing amino acids (Cys), which appeared to determine sensory taste. The third axis was correlated with Gly, Ala and Pro, which were thought to be unessential amino acids in nutritional quality evaluation.

3.4. Nutritional quality evaluation of DPM and DPMHs

The amino acid composition of DPM and DPMHs are shown in Table 4. The EAA composition was different between DPM and DPMHs. However, the total EAA content was not significantly ($p > 0.05$) different. The nutritional quality of DPM and DPMHs was evaluated by EAA score (Table 4). The data showed that DPM had a surplus of Phe + Tyr, Val, Ile and His, compared with FAO/WHO (1991) reference model. Enzymatic treatment made the contents of these EAAs closer to the reference standard. Moreover, the sulphur-containing amino acids as Met and Cys were very low in peanut, which were considered as major limited amino acids of peanut. Moreover, the enzymatic treatment led to products with different nutritional qualities. In CPE hydrolysate, the EAA score of Met + Cys increased from 0.720 to 0.970, while there was a slight decrease for that of commercial protease hydrolysates. This was probably due to the extraction of more Met-rich polypeptides during hydrolysis, which was in consistent with the results of Basha and Pancholy (1981). Lys was found to be the second limited amino acid in DPM protein, which was in agreement with the results of Bensaba, Frampton, Allen, and Hill (1958). The CPE treatment remarkably increased Lys content to improve the nutritional quality of DPMH. The remained EAAs of CPE hydrolysate were different to commercial protease hydrolysates. For CPE hydrolysate, the EAA content was close to the standard pattern, while the EAA contents of commercial protease hydrolysates were similar to that of DPM.

Meanwhile, the factor scores (Table 3) could be used to select additional pairs from these EAA scores. The first three factor scores were represented by a three-armed star. Each arm represented a different factor and the arm length was proportional to the factor score. The first factor score of CPE hydrolysate was higher than that of DPM protein and other hydrolysates. It indicated that the nutritional quality of CPE hydrolysate was the highest.

3.5. Sensory evaluation of DPMHs

The results of sensory evaluation based on descriptive analysis test by ten assessors are shown in Fig. 2. The hydrolysates showed a complex of bitter, umami, salty and full-bodied tastes. All the hydrolysates except for Protamex hydrolysate had high scores of bitter taste, whereas CPE hydrolysate obtained the highest scores of the other three indices.

The results of the relativity analysis are shown in Fig. 2, which showed that for bitter descriptors. There was significant difference between the bitter intensity of CPE hydrolysate and that of commercial protease hydrolysates ($p < 0.05$). According to Ney (1971), all the hydrolysates should not have bitter taste because all the Q -values were below 1400 calcs^{-1} (Table 4). Actually, the four DPMHs had obviously bitter taste, which was in consistent with the result of Adler-Nissen (1984), who found that the extrapolated Q -values was not proved and explained that the bitterness was caused by the presence and concentration of hydrophobic peptides formed during protein hydrolysis. Otherwise, the bitterness was probably controlled by different proteases. CPE and protamex were mixture of endo- and exoproteases, while Alcalase and papain were endoproteases. Endoproteases make nonpolar amino acid residues at the C-terminus of the peptide formed and result in relatively high bitterness. Exoproteases can selectively release N-terminal amino acid residues to debitter protein hydrolysates

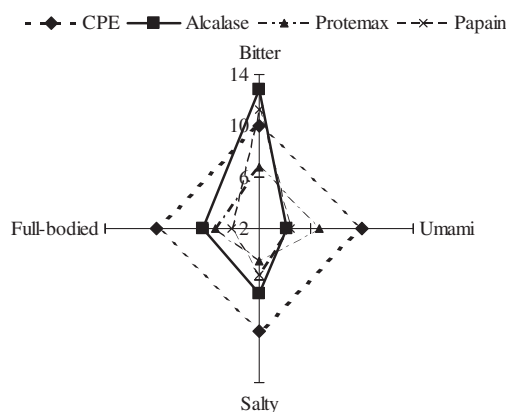
Table 3
Results of principal component analysis and the first three factor scores.

Component number	1	2	3
Eigenvalue	8.3	5.32	3.59
Cumulative %	46.1	75.5	95.6
Asp	0.27	−0.958	0.076
Glu	0.058	−0.989	−0.029
Ser	−0.952	0.041	−0.288
Gly	−0.284	−0.255	−0.914
His	−0.676	0.647	0.288
Arg	−0.613	0.72	−0.282
Thr	0.911	−0.306	0.277
Ala	0.46	0.312	0.823
Pro	−0.49	0.126	−0.799
Tyr	0.092	0.959	0.241
Val	0.728	0.526	0.438
Met	0.925	−0.286	0.198
Cys	0.102	0.881	0.275
Ile	0.849	0.226	0.38
Leu	0.786	0.078	0.579
Trp	0.882	−0.018	0.406
Phe	−0.829	0.321	−0.243
Lys	0.956	0.052	0.206
<i>Factor score</i>			
DPMP	−0.182	1.69	0.285
CPE	1.69	−0.347	0.48
Alcalase	−0.54	−0.027	−0.107
Protamex	−0.912	−0.942	0.986
Papain	−0.051	−0.372	−1.64

Table 4

Amino acid composition (g/100 g) and nutritional quality evaluation of DPMP and DPMHs*.

Amino acids	DPMP	Hydrolysates by different proteases				
		CPE	Alcalase	Protamex	Papain	
Asp	9.75 ± 0.60 ^a	11.8 ± 1.81 ^a	11.1 ± 1.40 ^a	11.7 ± 1.47 ^a	11.3 ± 1.57 ^a	
Glu	22.0 ± 3.79 ^a	24.2 ± 2.26 ^a	23.5 ± 2.09 ^a	24.8 ± 2.27 ^a	24.3 ± 1.97 ^a	
Ser	4.54 ± 0.41 ^a	2.22 ± 0.20 ^b	4.75 ± 0.30 ^a	5.02 ± 0.33 ^a	4.96 ± 0.29 ^a	
Gly	5.51 ± 0.45 ^a	5.41 ± 0.19 ^a	5.73 ± 0.24 ^a	5.61 ± 0.37 ^a	6.35 ± 0.51 ^a	
His	3.55 ± 0.41 ^a	2.43 ± 0.15 ^b	3.30 ± 0.36 ^{ac}	3.06 ± 0.32 ^{bc}	2.62 ± 0.26 ^{bc}	
Arg	12.7 ± 0.46 ^a	9.98 ± 0.56 ^a	11.5 ± 1.39 ^a	11.1 ± 1.25 ^a	11.7 ± 1.82 ^a	
Thr	2.94 ± 0.38 ^a	3.93 ± 0.33 ^b	2.97 ± 0.15 ^a	3.08 ± 0.17 ^a	3.03 ± 0.25 ^a	
Ala	3.94 ± 0.34 ^a	4.09 ± 0.30 ^a	3.69 ± 0.21 ^{ab}	3.74 ± 0.28 ^{ab}	3.22 ± 0.23 ^b	
Pro	4.83 ± 0.37 ^{ab}	4.21 ± 0.36 ^a	5.36 ± 0.15 ^b	4.50 ± 0.47 ^a	5.47 ± 0.45 ^b	
Tyr	4.89 ± 0.35 ^a	4.10 ± 0.37 ^b	4.00 ± 0.33 ^b	3.84 ± 0.24 ^b	3.83 ± 0.28 ^b	
Val	4.51 ± 0.31 ^a	4.69 ± 0.28 ^a	3.95 ± 0.39 ^a	3.78 ± 0.48 ^a	3.68 ± 0.42 ^a	
Met	1.35 ± 0.22 ^a	2.07 ± 0.36 ^b	1.27 ± 0.16 ^a	1.43 ± 0.21 ^a	1.46 ± 0.21 ^a	
Cys	0.46 ± 0.10 ^a	0.36 ± 0.08 ^a	0.32 ± 0.09 ^a	0.34 ± 0.09 ^a	0.33 ± 0.13 ^a	
Ile	3.49 ± 0.42 ^a	3.88 ± 0.39 ^a	3.43 ± 0.19 ^a	3.20 ± 0.42 ^a	3.17 ± 0.33 ^a	
Leu	6.10 ± 0.34 ^a	6.70 ± 0.96 ^a	5.60 ± 0.36 ^a	5.95 ± 0.34 ^a	5.57 ± 0.41 ^a	
Trp	0.850 ± 0.10 ^a	1.11 ± 0.15 ^a	0.860 ± 0.21 ^a	0.800 ± 0.20 ^a	0.760 ± 0.17 ^a	
Phe	4.88 ± 0.29 ^a	4.35 ± 0.40 ^a	5.06 ± 0.25 ^a	4.75 ± 0.29 ^a	4.79 ± 0.23 ^a	
Lys	3.68 ± 0.15 ^a	4.50 ± 1.01 ^a	3.65 ± 0.17 ^a	3.35 ± 0.33 ^a	3.50 ± 0.53 ^a	
EAAAs	31.4 ± 1.51 ^a	33.7 ± 2.37 ^a	30.1 ± 1.69 ^a	29.4 ± 1.19 ^a	28.6 ± 2.49 ^a	
Q-values (Cal/mol)	1077.0 ± 11.88 ^{ab}	1090.6 ± 37.50 ^a	1051.4 ± 15.07 ^{ab}	1018.5 ± 25.65 ^b	1024.5 ± 33.52 ^{ab}	
Umami Acids	51.0 ± 2.53 ^a	53.9 ± 3.26 ^a	53.1 ± 2.77 ^a	54.4 ± 0.68 ^a	53.8 ± 1.98 ^a	
EAA score						FAO/WHO 1991 pattern
Lys	0.63 ± 0.03	0.78 ± 0.17	0.63 ± 0.03	0.58 ± 0.06	0.60 ± 0.09	5.8
Thr	0.86 ± 0.11	1.16 ± 0.10	0.87 ± 0.04	0.91 ± 0.05	0.89 ± 0.07	3.4
Val	1.29 ± 0.09	1.34 ± 0.08	1.13 ± 0.11	1.08 ± 0.14	1.05 ± 0.12	3.5
Ile	1.25 ± 0.15	1.39 ± 0.14	1.23 ± 0.07	1.14 ± 0.15	1.13 ± 0.12	2.8
Leu	0.92 ± 0.05	1.01 ± 0.15	0.85 ± 0.05	0.91 ± 0.09	0.84 ± 0.06	6.6
Phe + Tyr	1.55 ± 0.10	1.34 ± 0.09	1.44 ± 0.08	1.36 ± 0.06	1.37 ± 0.08	6.3
Trp	0.77 ± 0.09	1.01 ± 0.13	0.78 ± 0.19	0.73 ± 0.18	0.69 ± 0.16	1.1
Met + Cys	0.72 ± 0.13	0.97 ± 0.16	0.64 ± 0.09	0.71 ± 0.12	0.72 ± 0.09	2.5
His	1.87 ± 0.21	1.28 ± 0.08	1.74 ± 0.19	1.61 ± 0.17	1.38 ± 0.14	1.9

* The values in the same row followed by different letters are significantly different ($p < 0.05$).**Fig. 2.** The sensory evaluation of DPMHs prepared by CPE and other commercial proteases.

(Saha & Hayashi, 2001). The CPE hydrolysate had more small peptides than papain hydrolysate, which might be responsible for the low bitterness of CPE hydrolysate.

Based on the amino acid composition, the contents of umami amino acids of four DPMHs were not significantly different ($p > 0.05$) (Table 4). However, umami taste of final hydrolysate with CPE was obviously stronger ($p < 0.05$), followed by Protamex, while Alcalase and Papain had relatively low umami taste. It was probably due to the CPE and Protamex hydrolysates had much lower peptides and free amino acids to enhance the hydrolysate umami taste. As reported by Okumura, Yamada, and Nishimura (2004), the addition of fraction (MW 0.5–1 KDa) can enhance umami and saltiness.

ANOVA showed that the CPE hydrolysate exhibited significantly higher salty taste ($p < 0.05$). Tamura et al. (1988) have pointed out that some peptides which contain basic amino acids, can produce salty taste. Alcalase has significant ($p < 0.05$) stronger salty taste than Protamex and papain, This may be due to the addition of NaOH to increase pH during operation.

The full-bodied taste is important for the final products. As shown in Fig. 2, enzymatic treatment could enhance the full-bodied taste of hydrolysate. CPE was the most efficient protease for enhancing the full-bodied taste, followed by protamex and Alcalase.

The above results showed that the CPE hydrolysate had good comprehensive indices of taste, and was suitable to be used in condiments.

4. Conclusions

Based on the results of this work, CPE had high hydrolysis efficiency on DPM than the commercial proteases. The nutritional qualities of DPM proteins could be apparently enhanced by CPE treatment. Furthermore, the CPE hydrolysate had better sensory taste than other hydrolysates. The enzyme composition of CPE and their biochemical characteristics are still not clear. Further work on purification and identification of CPE is worthy to be carried out.

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