Preparation of High Fischer Ratio Oligopeptide by Proteolysis of Corn Gluten Meal

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Abstract

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A method to obtain an oligopeptide with high Fischer ratio is described. Corn gluten meal (CGM) was hydrolysed with Alcalase 2.4L using a two-step hydrolysis. In the first-step hydrolysis, the enzyme reaction conditions for hydrolysing CGM were optimised by using the orthogonal experimental design, while pH = 8.0, temperature = 55°C, enzyme to substrate ratio (3:97, w/w), and the substrate concentration = 5% were identified as the optimum conditions, under which up to 11.62% degree of hydrolysis (DH) could be obtained. The hydrolysate was then fractionated by ultrafiltration using a membrane with the molecular cutoff of over 10 kD at 20 kPa. For the second-step hydrolysis, the filtrate was adjusted to pH 6.0, then papain was added at 50°C and the mixture was maintained for 3 hours. The hydrolysate was obtained after inactivating papain and centrifuging. Then the salt (mainly NaCl) in the hydrolysate was removed with an ion exchange resin at the speed of 8 times bed volume per hour, and aromatic amino acids were removed through absorption by active carbon. By using Sephadex G-25 gel filtration chromatography, a peptide mixture with low molecular weights between 1000 and 1300 was obtained. Finally, tests on amino acid composition and free amino acid concentration of oligopeptide solution showed that the oligopeptide had a high Fischer ratio of 34.71 and the yield of 11.59%.

Keywords: corn gluten meal; enzyme hydrolysis; high Fischer ratio oligopeptide; CGM; amino acid; peptide

The peptide with high levels of branched chain amino acids (BCAA) and low levels of aromatic amino acids (AAA) is called high Fischer ratio oligopeptide. High Fischer ratio oligopeptide is an active peptide with a low molecular weight, derived from various food proteins.

Patients with severe hepatic disease generally have an amino acid imbalance characterised by low levels of BCAA and high levels of AAA in their systemic blood (Tanimoto *et al.* 1991). It has been reported that an increase in AAA levels in the brain leads to a decrease in the normal neurotransmitters and an increase in the neurologically inactive phenylethanolamine and octopamine (Fisher & Baldessarini 1971), and that BCAA intake improves the plasma amino acid balance. Therefore,

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high Fischer ratio oligopeptides help to mitigate the symptoms of hepatic encephalopathy. Consequently, there is a market need for the development of an oligopeptide mixture with a high Fischer ratio.

For this reason, several studies have been carried out. Adachi *et al*. (1991) successfully produced a high Fischer ratio oligopeptide from a casein hydrolysate treated consecutively with thermolysin and papain, but the Fischer ratio obtained was only about 5. Bautista *et al*. (1996) reported a method to obtain low molecular weight hydrolysates with a high Fischer ratio from sunflower protein with a high Fischer ratio of 20.47 at the yield of 24.8% on the nitrogen basis for patients with liver failure. The method involved hydrolysing the sunflower protein solution with kerase in a pH-stat at constant pH of 7.5 until the degree of hydrolysis (DH) of 16.5 was achieved, and the hydrolysate was fractionated by ultrafiltration using membranes with cutoffs of 30 000 and 5000 (Bautista *et al*. 1996). Mannheim and Cheryan (1992) found that functional properties of proteins in corn gluten meal (CGM) can be improved by enzyme hydrolysis with Alcalase 2.4L, the degree of hydrolysis (DH) value obtained was 27.7% and protein conversion rate was 35.9%. Tanimoto *et al*. (1991) found that when zein was hydrolysed first with alkalophilic proteinase and then with actinase to liberate aromatic amino acids, the final product with a Fischer ratio of 20.0 at the yield of 56% on the nitrogen basis could be obtained.

CGM is a by-product of the corn processing industry which is normally used as animal feed. In China, CGM is mainly used to extract zein, to prepare corn gluten foaming powder, to extract maize yellow pigment, to produce amino acids, and to manufacture the edible corn gluten meal. As CGM has a high level of BCAA (up to 12.68%, as indicated in Table 1), its Fischer ratio is also high, which makes it feasible for using CGM to produce high Fischer ratio oligopeptide.

Table 1. Amino acid composition of CGM and zein

AA	CGM (%)	Zein (%)	Type of AA
Ile	2.05	1.93	BCAA
Leu	8.24	1.06	BCAA
Lys	0.96	0.03	
Met	1.05	0.45	
Cys	0.56	0.40	
Phe	3.09	3.90	AAA
Tyr	2.31	2.74	AAA
Thr	1.52	1.38	
Trp	0.20	0.12	AAA
Val	2.39	1.64	BCAA
Asp	3.21	2.60	
Ser	2.51	2.95	
Glu	12.26	7.10	
Gly	1.36	1.40	
Ala	4.81	3.88	
His	0.87	0.49	
Arg	1.56	0.98	
Pro	3.00	4.69	
BCAA	12.68	4.63	
AAA	5.60	6.76	

Despite the above studies, in order to further increase the productivity of the high Fischer ratio oligopeptide from CGM, a procedure consisting of two-step hydrolysis, ultrafiltration, salt removal, and active carbon absorption was used in the current study. This procedure combined advantages of the approaches used in the previous studies. Six different types of enzymes including Alcalase 2.4L, Nutrase, Neutral AS1398, Alcalase (domestic), proteolytic enzyme, and pepsin were used to identify the most appropriate enzyme, so that a high Fischer ratio oligopeptide from CGM could be produced.

MATERIALS AND METHODS

Materials

CGM was purchased locally (Longgong Corn Gluten Ltd, Harbin, China), its amino acid (AA) composition is shown in Table 1. Six different enzymes used were Alcalase 2.4L (Novo Co., Bagsvaerd, Denmark) with the activity of 7.14×10^4 U/g; Nutrase (Novo Co., Bagsvaerd, Denmark) with the activity of 2.08×10^4 U/g; Nutrase AS1398 (Genencor Biotechnology Co., Wuxi, China) with the activity of 1.17×10^4 U/g; Alcalase (Jinchao Ltd, Haining, China) with the activity of 4.95×10^4 U/g; proteolytic enzyme (Longbo Biotechnology Ltd, Nanning, China) with the activity of 4.01×10^4 U/g; and pepsin (Huashun Biotechnology Ltd, Shanghai, China) with the activity of 1.60×10^5 U/g. Papain (Guangzhou Enzyme Ltd, Guangzhou, China) used in second-step hydrolysis, active carbon (Pengcheng Active Carbon Ltd, Chengde, China), strong alkali anion exchange resin 201 × 7 (717) and strong acidic cation exchange resin 001 × 7 (732) (Xinxi Reagent Co., Shenyang, China) were also obtained locally. Other chemicals used were of analytical grade.

Analysis

Protein content (N × 6.5) was determined by the micro-Kjeldahl method (Anonymous 1980). The fat content was determined by the extraction of samples with petroleum ether (Anonymous 1984). The moisture in the samples was determined by a two-step drying scheme (Anonymous 1980).

The protein conversion rate was measured by the trichloroacetic acid method (Mannheim & Cheryan 1993), which was calculated as the total nitrogen (N) soluble in 10% trichloroacetic acid (TCA) in the reaction mixture as the percentage of total nitrogen present in the unhydrolysed protein suspension, i.e., the protein conversion rate could be calculated from the following equation:

$$X = (P - P_0)/(S_0 - P_0) \times 100\% \quad (1)$$

where:

X – protein conversion rate (%)

P – product concentration (nitrogen in TCA-soluble fraction of hydrolysate)

P_0 – initial "product" concentration (nitrogen in TCA-soluble fraction of unhydrolysed substrate)

S_0 – initial substrate concentration (nitrogen in unhydrolysed substrate)

The degree of hydrolysis (DH) is defined as the ratio (expressed as %) between the number of hydrolysed peptide bonds over the total number of bonds available for hydrolysis (Bautista *et al.* 2000) which was measured by ninhydrin photometric method (Panasiuk *et al.* 1998). DH was calculated from the following equation:

$$\text{DH (\%)} = \frac{h}{h_{\text{hot}}} \times 100\% \quad (2)$$

where:

h – number of hydrolysed peptide bonds (given in milli-molar/gram protein)

h_{hot} – total number of peptide bonds in the protein substrate = 9.2 milli-molar/gram protein

The salt content was measured based on chloride determination (Anonymous 1980). The amino acid composition and free amino acids in the fractions were determined with a high performance amino acid automatic analyser system (Model 835-50, Hitachi Ltd, Japan).

Pretreatment of CGM

In order to improve hydrolysis rates, CGM was subjected to the pretreatment proposed by Mannheim and Cheryan (1992). The procedure for the pretreatment was as follows: the reaction mixture (different concentrations of CGM solution) was adjusted to pH 9.0, sodium sulfite (1.5 g/l of reaction mixture) was then added, and the mixture was incubated at 50°C for 60 minutes.

Enzymatic hydrolysis of corn gluten meal

Choosing optimal conditions for proteolysis. For choosing the most appropriate enzyme, preliminary hydrolysis assays were performed. 5 g CGM was added into 100 ml distilled water to make the reaction mixture, then each protease was added to the mixture at the activity of 2000 U/g, respectively. The mixture (5%, w/v) was hydrolysed for 5 h at the proper temperature and pH recommended by the manufacturers. Then the DH, protein conversion rate, and the content of free amino acids were determined, so as to select the most suitable protease. The selected protease was used for the following hydrolysis of CGM consisting of two-steps.

First-step hydrolysis. The first-step hydrolysis was to determine the optimum enzyme reaction conditions for the enzyme chosen. For this purpose, a 4-factor 3-level ($L_9 3^4$) orthogonal experimental design was employed. The factors selected were pH (Factor A), the reaction temperature (Factor B), the concentration of enzyme to substrate ([E]/[S] w/w) (Factor C), and the concentration of substrate ([S]) (Factor D). Table 2 shows the three levels for each factor. The choice of the levels for each factor was based on the previous mono-factor experiments. The degree of hydrolysis was chosen as the index.

Various experiments for hydrolysis of CGM were conducted according to Table 2. During hydrolysis, NaOH was continuously added to the mixture in order to maintain the required pH. After 4 h of hydrolysis, pH was adjusted to 6.0 by adding 2 mol/l HCl to precipitate unhydrolysed proteins. The reaction mixture was then heated to 90°C for 15 min to inactivate the enzyme, and immediately after the enzyme inactivation, the mixture was cooled to room temperature. The mixture was centrifuged at 4000 rpm for 20 min, the supernatant was finally collected as the hydrolysate and its volume was measured.

Second-step hydrolysis. As the hydrolysate obtained in the first-step hydrolysis still contained unhydrolysed proteins and poly-peptides with high molecular weights, ultrafiltration using a NMWL10000 membrane (an ultrafiltration membrane that can retain molecules with molecular weights of over 10 kD) at 20 kPa and 40°C was performed to remove the large molecular substances.

To release AAA, the ultrafiltrate (100 ml) was adjusted to pH 6.0. 0.1 g papain was added and the second hydrolysis was then conducted at 50°C for 180 minutes. Similar to the first-step hydrolysis, the sample was adjusted to pH 2.5 and maintained at 90°C for 15 min to deactivate the enzyme, and then cooled to room temperature. Finally, samples were centrifuged at 4000 rpm for 20 min, and the supernatant was collected as the second hydrolysate.

Desalting and active carbon absorption

The hydrolysates contained many salts (mainly NaCl) due to the addition of NaOH and HCl used to adjust pH. As excessive salts have negative effects on the products and human health, it is necessary to remove them. Therefore, the ion-exchange method was selected for desalting, and the strong alkali anion exchange resin 201 × 7(717) and strong acidic cation exchange resin 001 × 7(732) were used due to their good effectiveness and low costs, and the desalt ratio (DR) and protein recovery ratio (PRR) of the hydrolysates were measured. The desalt ratio was calculated from the following equation:

$$DR\ (\%) = 1 - \frac{S_2 V_2}{S_1 V_1} \times 100\% \quad (3)$$

where:

S_1 – salt concentration of hydrolysate (g/l)

V_1 – volume of hydrolysate (l)

S_2 – salt concentration of effluent liquid (g/l)

V_2 – volume of effluent liquid (l)

The protein recovery ratio can be determined by the equation below:

Table 2. Factors and levels of orthogonal experimental design $L_9(3^4)$

Level	Factor A [pH]	Factor B [Reaction temperature (°C)]	Factor C [E]/[S]	Factor D [S]
1	8.0	55	2	5.0
2	8.5	60	3	7.5
3	9.0	65	4	10.0

Table 3. Protein conversion rate and DH of CGM hydrolysed by different proteases

Protease	pH	T (°C)	Protein conversion rate (%)	DH (%)
Alcalase 2.4L	8.5	55	78.5	10.3
Nutrase	6.5	45	5.80	2.5
Nutrase AS1398	7.0	37	47.5	6.8
Alcalase (domestic)	10.0	50	70.8	9.7
Proteolytic enzyme	6.5	55	14.0	4.2
Pepsin	2.0	25	3.86	2.0

$$\text{PRR (\%)} = \frac{C_2 V_2}{C_1 V_1} \times 100\% \quad (4)$$

where:

C_1 – protein concentration of hydrolysate (g/l)

C_2 – protein concentration of effluent liquid (g/l)

The solution hydrolysed by papain in the second-step hydrolysis contained enormous amounts of free AAA, which should be separated to obtain a high Fischer ratio oligopeptide. An active carbon absorption column was employed to absorb AAA selectively. The absorption experiment was carried out at the temperature of 25°C~35°C and pH of 2.0~3.0. In order to determine the content of AA in the solution after active carbon absorption, an ultraviolet visible (UV) spectrophotometer (754PC, Shanghai Spectrum Instruments Ltd, China) was used to detect AAA at 260 nm and BCAA at 220 nm.

Molecular weight distribution was determined by means of a high performance gel filtration column (TSK G2000 SW, 7.5 mm × 30 cm, Tosoh Corp., Tokyo, Japan). The high performance liquid chromatography (HPLC) system was coupled to a Spectronic 1001 Ultraviolet Visible Spectrophotometer (as the detector) (Rochester, NY) and an IBM (Armonk, NY) PC computer (for data acquisition). Dried samples of the reaction mixtures, ultrafiltration retentates and permeates were suspended in a phosphate buffer mobile phase (0.067 mol/l, pH 7.4), consisting of 19% (v/v) monobasic sodium phosphate monohydrate and 81% dibasic sodium phosphate at the concentration of 10 mg/ml. The highly turbid solutions were centrifuged at 1500 rpm for 10 min and filtered through a 0.45 μm filter. Samples were then injected into a 20 μl injection loop of the injector. The operating conditions for the HPLC system were as follows: ambient temperature of 23 ± 2°C; flow rate of 1.0 ml/min and absorbance at 205 nm (Mannheim & Cheryan 1992).

RESULTS AND DISCUSSION

Hydrolysis

Choosing of protease. Table 3 shows the results of the protein conversion rate and DH of CGM hydrolysed by the six proteases. In the CGM-Alcalase 2.4L batch hydrolysis, the protein conversion rate was 78.5% and the DH value obtained was 10.3%. In comparison, in the other proteinase batch hydrolysates, the protein conversion rates were between 3.86% and 70.8%, while the DH values obtained were within the range of 2.0–9.7%. Therefore, Alcalase 2.4L was selected because it appeared to possess the highest activity. In a separate study (Mannheim & Cheryan 1992), Alcalase 2.4L was also used to hydrolyse CGM with protein conversion rate of 35.9% and DH value of 27.7%.

The higher DH of CGM-Alcalase 2.4L batch hydrolysis was due to the better solubility of CGM in alkaline solution, thereby the enzyme was more accessible to the protein and hydrolysis was easier (Mannhelm & Cheryan 1992). Furthermore, Alcalase 2.4L is an alkaline serine protease that attacks peptide bonds randomly, but has preference for the peptide bonds adjacent to hydrophobic and/or aromatic amino acids, such as tyrosine,

Table 4. Ratio of BCAA and AAA over total AA (%)

Protease	Free BCAA	Free AAA
Alcalase 2.4L	19.7	36.2
Nutrase	21.6	29.5
Nutrase AS1398	30.2	20.1
Alcalase (domestic)	28.1	26.4
Proteolytic enzyme	26.6	18.1
Pepsin	19.3	32.8

phenylalanine, and tryptophan. It can cleave peptide bonds inside the peptide linkage to form two peptides, and isolate the native proteins and peptides (Mannhelm & Cheryan 1992). On the other hand, the DH of pepsin to CGM was the lowest, and although pepsin could quickly hydrolyse the peptide linkage formed by phenylalanine, zein is difficult to disperse in water at an acidic pH, therefore the use of pepsin was not recommended (Tanimoto *et al*. 1991).

Table 4 compares the free amino acid compositions generated by the hydrolysis of CGM with the six proteases. As indicated in Table 4, from all the six enzymes, Alcalase 2.4L creates a relatively low free BCAA content (19.7%) and a relatively high free AAA content (36.2%) in the resulting hydrolysate. As the purpose of the current study was to obtain the BCAA-rich and AAA-poor peptide solution, BCAA should be maintained while AAA should be removed in the experiment. Therefore, Alcalase 2.4L was selected as the most suitable enzyme in the subsequent experiments.

Optimising hydrolysis conditions. As the conditions for protease hydrolysis have a great influence on the peptide concentration in hydrolysates, for achieving a high hydrolysate of protein the hydrolysis parameters such as pH, temperature, [E]/[S] and [S] should be optimised (Ader-Missen 1979).

Table 5 shows the results of the $L_9(3^4)$ orthogonal experiment design as detailed in Table 2. From Table 5, it can be observed that the effect of the four factors on DH is pH > temperature > [E]/[S] > [S], and the optimum reaction condition was identified as pH = 8.0, temperature = 55°C, [E]/[S] = 3:97 (w/w), and [S] = 5%, under which up to 11.62% DH could be obtained. In a previous study (Mannheim & Cheryan 1992), a higher DH of 27.7% was achieved, probably due to that the enzyme and the CGM used came from different sources.

Second-step hydrolysis. The use of Alcalase 2.4L in the first step hydrolysis led to peptides with AAA at the C-terminus. Therefore, the ultrafiltrate was hydrolysed again. Papain was used in the second-step hydrolysis, as it can cleave different peptide bonds, leading to the release of AAA so that the hydrolysates have a higher Fischer ratio. The AA composition of the hydrolysates provided by papain as compared with that of the first-step

Table 5. Design of the orthogonal experiment and results of Alcalase hydrolysis

No. of experiment	pH	T (°C)	[E]/[S] (%)	[S] (%)	DH (%)
1	1	1	1	1	11.09
2	1	2	2	2	10.04
3	1	3	3	3	10.44
4	2	1	2	3	8.72
5	2	2	3	1	9.00
6	2	3	1	2	5.25
7	3	1	3	2	9.52
8	3	2	1	3	8.15
9	3	3	2	1	8.54
K_1	31.56	29.34	24.48	28.62	
K_2	22.98	27.18	28.95	24.81	
K_3	26.22	24.24	27.3	27.3	
k_1	10.52	9.78	8.16	9.54	
k_2	7.66	9.06	9.65	8.27	
k_3	8.74	8.08	9.10	9.10	
R	2.86	1.70	1.49	1.27	

K – sum of DH at each level; k – average of K at each level; R – difference between maximum k and minimum k

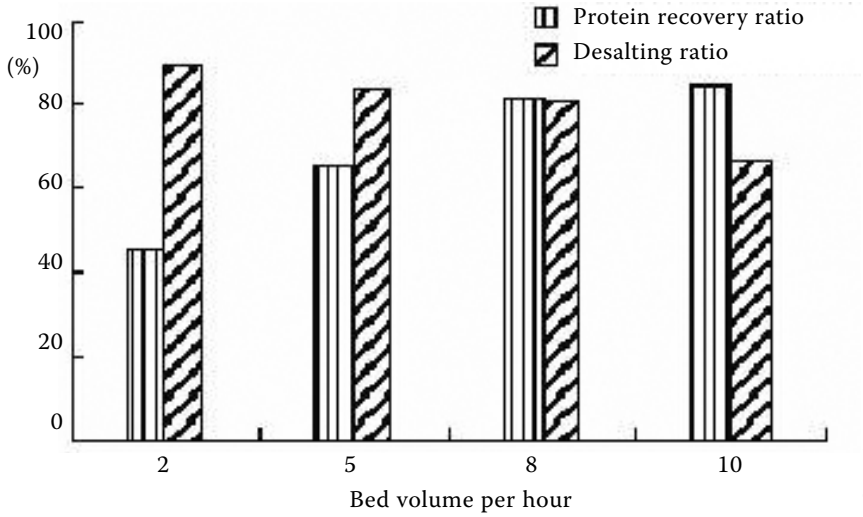

Figure 1. Comparison of desalinisation as affected by different velocities

hydrolysis is shown in Table 6, indicating that the papain hydrolysate contained more free phenylalanine and tyrosine. Lopes *et al.* (2005) also confirmed the effect of papain on the release of phenylalanine.

Desalting and active carbon absorption

Figure 1 shows the results of the desalting process as affected by the ion exchange rate measured in the bed volume per hour (BV/h). It can be seen

Table 6. Comparison of variety and quantity of the composing amino acid (AA) and free amino acid (FAA) hydrolysed with papain (mg/ml)

Amino acid	Composing AA	Free AA (first hydrolysis)	Free AA (second hydrolysis)
Cys	0.29	0.20	0.09
Met	1.20	0.09	0.06
Asp	2.96	–	–
Thr	1.63	0.12	0.73
Ser	2.45	0.11	0.19
Glu	10.72	0.09	0.20
Gly	1.29	0.05	0.04
Ala	4.47	–	0.04
Cys	0.51	0.41	0.05
Val	2.13	0.36	0.27
Met	0.34	0.06	0.26
Ile	1.70	0.20	0.23
Leu	7.55	0.13	0.19
Tyr	2.17	0.50	0.66
Phe	2.57	0.07	0.71
Lys	0.82	0.06	0.04
His	0.98	–	–
Arg	1.40	–	–
Total	45.18	2.45	4.21

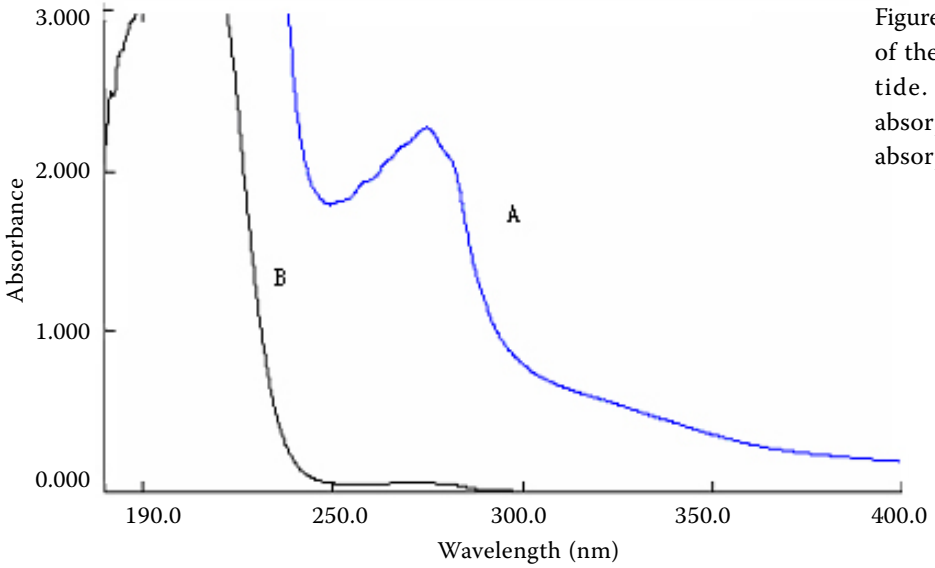

Figure 2. The UV-scanning spectrum of the high Fischer ratio oligopeptide. A – before active carbon absorption; B – after active carbon absorption

that the greater the velocity, the higher the desalted protein recovery ratio, and the lower the desalting ratio. At ion exchange velocity of 8 times the bed volume per hour, the protein recovery ratio was 81.67%, the desalting ratio was 80.95%. In another research (Zhao 1997), the ion-exchange method was used in desalting the hydrolysates of soy protein with the protein recovery ratio being 91.5% and the desalting ratio being 90.3%, which is close to the current result.

Table 7 shows the effect of different active carbon concentrations on the level of AAA absorption. When the active carbon-to-CGM ratios were 1:12, 1:10 and 1:8, the absorption of AAA was relatively high (hence the remaining free AAA in the solution was low) as shown by the low OD_{260} in Table 7, and the 1:12 batch had the least BCAA absorption by the active carbon (thus the highest OD_{220} in Table 7), so the ratio of 1:12 batch was selected for achieving high carbon absorption of free AAA.

Table 7. Effect of different active carbon concentrations on absorption of AAA

Active carbon-to-material ratio	OD_{260} of absorption solution (for free AAA)	OD_{220} of absorption solution (for BCAA)
1:8	0.061	1.065
1:10	0.057	1.523
1:12	0.061	1.959
1:20	0.090	0.757
1:30	0.133	0.951
1:40	0.169	1.076

After active carbon absorption, the sample was subjected to ultraviolet (UV)-scanning, and the

Table 8. Amino acid composition of high Fischer ratio oligopeptide (mg/ml)

Amino acid	Free AA composition	Free AA concentration
Cys	0.13	0.04
Met	0.37	–
Asp	1.17	0.03
Thr	0.45	0.02
Ser	0.97	0.02
Glu	2.31	0.02
Gly	0.46	0.09
Ala	2.57	0.11
Val	0.77	0.11
Ile	0.27	0.10
Leu	1.90	0.12
Tyr	–	–
Phe	0.11	0.08
Lys	0.37	0.13
His	0.21	–
Arg	0.29	–
Pro	0.58	–
Total	13.55	0.87
Proportion (%)	–	6.42

Table 9. Flow-out volumes of substances with standard relative molecular masses

Name	Potassium chromate	GSSH	Vitamin B_{12}	Lysozyme
Molecular weight	192	307	1300	14400
Elution volume	34.6	31.2	28.4	15.8

result is illustrated in Figure 2, indicating that active carbon was efficient to absorb aromatic amino acid because the UV-absorbance of the mixture at 260 nm was reduced to almost zero. Lopes *et al*. (2005) also stated that the active carbon was efficient to remove Phe from skim milk powder hydrolysates, and the removal rate of Phe was between 96% and 99%.

Amino acid composition analysis of high Fischer ratio oligopeptide

Based on the above results, the procedure for preparing a high Fischer ratio oligopeptide was developed. In order to verify the procedure, the following experiment was carried out: 50 g CGM that had been pretreated was mixed with 1000 ml distilled water. The sample was adjusted to pH 8.0 with 6 mol/l NaOH, then Na_2SO_3 (1.5 mg/ml of reaction mixture) was added. The sample was stirred at 55°C for 60 minutes. Alcalase 2.4L was then added at [E]/[S] ratio of 3%, stirred at 55°C for 4 hours. Finally, the sample was heated to 90°C to deactivate the enzymes. After deactivation, the sample was centrifuged at 4000 rpm for 20 min and the supernatant was ultrafiltered through the NMWL10000 membrane at 20 kPa. The filtrate obtained was adjusted to pH 6.0. After adding papain, the sample was maintained at 50°C for 3 hours. The enzyme was deactivated by adjusting the pH to 2.5, then the sample was centrifuged to remove the residue. The second-step hydrolysates obtained were desalted using ion exchange resin at 8 times the bed volume per hour. Finally, the active carbon (active carbon-to-CGM ratio was 1:12) was used to remove AAA, and the high Fischer ratio oligopeptide solution was thus obtained.

Table 8 lists the amino acid composition of high Fischer ratio oligopeptide (mg/ml). The free amino acid concentration was 6.42%. The Fischer ratio of oligopeptide (the molar ratio of BCAA to AAA) was 34.71, and the productivity of the high Fischer ratio oligopeptide (the ratio of the protein content of high Fischer ratio oligopeptide to the mass of CGM) was 11.59%.

The high Fischer ratio oligopeptide obtained from CGM contained various peptides, a few free amino acids (FAA), and ash, etc. The elution volume of different standard substances with known molecular weights was determined and the results are shown in Table 9. Based on the elution curve obtained for the high Fischer ratio oligopeptide, the elution volume was determined to be 28.9 ml, which corresponded to the molecular weight in the range of 1000–1300 Da, with little free amino acids and unhydrolysed proteins.

CONCLUSIONS

Alcalase 2.4L can hydrolyse corn gluten meal to produce an oligopeptide with a high Fischer ratio. The reaction conditions of CGM hydrolysing optimised with orthogonal experimental design as pH 8.0, temperature 55°C, [E]/[S] 3:97 (w/w), and [S] 5%, under which conditions up to 11.62% DH could be obtained. With the two-step hydrolysis procedure, ultrafiltration, ion exchange and active carbon absorption, the oligopeptide obtained had a high Fischer ratio of 34.71 and a high yield of 11.59%, with the molecular weight within the range of 1000–1300 Da.

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