

Research Article

Study on Hydrolysis Conditions of Flavourzyme in Soybean Polypeptide Alcalase Hydrolysate and Soybean Polypeptide Refining Process

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Abstract: Soybean protein Alcalase hydrolysate was further hydrolyzed by adopting Flavourzyme as hydrolytic enzyme. The optimal hydrolysis conditions of Flavourzyme was that pH was 7.0 at temperature 50°C and E/S(ratio of enzyme and substrate) was 20LAPU/g. Bitterness value was reduced to 2 after Flavourzyme hydrolysis reaction in optimal hydrolysis conditions. The change of molecular weight distribution range from Alcalase hydrolysate to Flavourzyme hydrolysate was not obvious. DH (Degree of hydrolysis) of soybean protein hydrolysate was increased to 24.2% which was improved 3.5% than Alcalase hydrolysate. Protein recovery proportion was increased to 73.2% which was improved 0.8% than Alcalase hydrolysate. Soybean polypeptide Flavourzyme hydrolysate was decolorized with activated carbon which optimal dosage was 1.2% solution amount (w/w). Anion/cation exchange process was used in the desalination processing of soybean polypeptide. Ratio of anion resin and cation resin was 2:3(V/V). The volume of hydrolysate processed was 5 times as the volume of anion resin. Ash content of soybean peptide solution reduced to 2.11% (dry basis), salinity decreased by 86% after desalination processing.

Keywords: Activated carbon decolorization, alcalase hydrolysate, degree of hydrolysis, desalination process, flavourzyme, ion exchange resin

INTRODUCTION

Soybean protein is tasteless, mainly because molecular weight of soybean protein is very large, also because soybean protein molecules are close globular molecules structure which configuration is complex, most of their hydrophobic group are wrapped in molecular internal, hydrophobic group can't contact the taste bud in space. So we can't feel bitter of soybean protein. However, when the soybean protein is hydrolyzed by protease, their globular structure is destroyed, hydrophobic amino acid group that was wrapped in internal before hydrolysis exposes (Li *et al.*, 2012). Then hydrophobic group contacts taste bud, so bitterness can be tasted.

Debitterizing of soybean isolate protein is to remove hydrophobic amino acid group of the product because the hydrophobic group is source of bitter (Izawa, 2007). Debitterizing methods can be divided into two kinds which are physical chemical debitterizing methods and enzymatic debitterizing methods. Enzymatic debitterizing can also be divided into two kinds which are ammonia peptide enzymatic and carboxy peptide enzymatic, amino peptidase debitterizing methods were studied more (Paris, 2003; Lee, 2006). In this study one amino peptidase

enzymatic debitterizing method was adopted because of mild conditions, less nutrition loss of hydrolysate, also because enzymatic debitterizing reaction process is easy to control and machining features of the hydrolysate is not changed.

Alcalase Hydrolysate of soybean separation protein has a certain bitter taste. The bitter that was produced in hydrolysis process largely limits its application in practical production and the acceptability of products. Hydrophobic amino acids groups of bitter peptide in soybean protein hydrolysate generally located in the end of peptides, so specific enzyme can be used in hydrolysate processing to achieve effective resection of hydrophobic amino acid. After excision of hydrophobic amino acids, bitter peptide bitterness declined distinctly and the bitter taste of free hydrophobic amino acid is much lower than the original bitter peptide (Murray *et al.*, 2009). Flavourzyme is a kind of compound enzyme which is composed of the incision enzyme and the circumscribed enzyme. Effective resection of hydrophobic amino acid from protein end can be achieved by using this mixed enzyme. In this study Flavourzyme was used as hydrolytic enzyme to achieve further hydrolysis on soybean protein protease Alcalase hydrolysate, in order to study the down effect to the bitterness of soybean polypeptide of Flavourzyme.

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On the other hand soybean polypeptide mixture was decolorized using activated carbon adsorption decolorization method in this study. Activated carbon adsorption method with low cost and high safety that operation is simple can achieve good decolorization effect. At the same time, part of hydrophobic amino acids can be adsorbed through the adsorption effect of active carbon, so hydrolysate taste is improved obviously (Pu and Liang, 2012).

In addition, hydrolysate is also needed to carry on desalination processing to eliminate the high salinity in order to achieve good taste of product. In this study ion exchange resin was adopted to make desalination processing to soybean protein hydrolysate. Through the experiment desalting effect of ion exchange resin to hydrolysate and the role in the desalination was studied.

Finished product of soybean polypeptide is general processed into solid powder because powder is easy to save. Concentration of soybean protein solution before hydrolysis was 8%. Concentration of the soybean protein hydrolysate after centrifugal separation, decolorizing processing and desalination processing was 5% or so. However, when concentration was too low, drying process was hard to make, so hydrolysate was first for concentration before drying process. Because the soybean peptide solution was easy to make Browning reaction when the solution was heated, too high temperature can cause serious Browning in product and Browning reaction limits its application. Low temperature vacuum concentration was adopted to avoid products Browning reaction. Hydrolysate concentration was concentrated to 35% and then drying process was made by using the spray drying equipment.

EXPERIMENTAL

Materials: Soybean separation protein powder which protein content was 91% was provided by Shandong Jinan Guanli science and trading company.

Alkaline protease Alcalase 2.4 L which labeling enzyme activity was 2.4 Au/g was provided by Jinan Baitai enzyme preparation co. LTD.

Flavourzyme which labeling enzyme activity was 500 LAPU/g was also provided by Jinan Baitai enzyme preparation co. LTD.

Activated carbon powder was purchased from Shanghai Meibao company group. Both 732 cation exchange resin and 711 anion exchange resin were purchased from Shanghai biological engineering company.

Hydrolysis method of soybean polypeptide alcalase hydrolysate by flavourzyme: Soybean separation protein powder→dissolving and pretreatment→Alcalase hydrolysis→enzyme deactivation 15 minutes at 85°C→adjusting appropriate temperature and pH after cooling→Flavourzyme hydrolysis→enzyme deactivation→cooling to below 50°C→adjust pH to 4.2→supernatant was gained from centrifugal separation.

Optimization of flavourzyme enzymolysis reaction conditions: Soybean separation protein powder was first hydrolyzed by Alcalase, so Flavourzyme hydrolysis conditions of the substrate concentration had been determined. In order to improve the production efficiency reaction time was control in 3 h inside, different conditions including enzyme concentration, reaction temperature and pH and so on were chosen as influence factors on soybean protein hydrolysis coarse liquid further hydrolysis to determine optimal conditions of Flavourzyme debitterizing to soybean protein Alcalase hydrolysate.

Bitter evaluation method: Bitter evaluation standard was according to reference (Pu and Liang, 2012). It was that 1 mL soybean peptide solution sample was diluted to just can be tasted the bitter taste so far, diluted multiples was regarded as bitter taste value. The average of diluted multiples was as the final bitter taste value.

Activated carbon decolorizing method: Supernatant fluid from centrifugal separation was preheated to 50°C, activated carbon powder which weight ratio is 1 to 2% of the supernatant was added with stirring and constant temperature. Filtration separation was made after stirring 60 min. Residue was washed with water which amount was 10 times as activated carbon. After filtration each filtrate was mixed as soybean polypeptide clear liquid after activated carbon adsorption decolorization.

Ion exchange desalting method: The soybean peptide solution after activated carbon decolorizing flowed through 732 cation exchange resin column to remove Na⁺ and other cations and then flowed through the 711 anion exchange resin column to removal Cl⁻ and other anions. Finally residual solution in anion exchange resin column was eluted with no salt water. Velocity of desalting process with constant temperature 50°C was 6 mL/min. Diopter of effluent was measured to judge the outflow of soybean polypeptide. The column effluent was received which diopter was greater than 0. Conductivity of the liquid was measured to judge desalting effect. Finally the ash content of soybean peptide product was determined to inspect desalting effect.

Anion and cation exchange resin need activation regeneration treatment (Tao, 2012) before using. The first step was that foreign body in resin was rinsed clean with water and the resin was soaked for 12 h or so to make it fully adsorb water and expand. The second step was that the resin was soaked 4 h above with about 10% of salt water which was 2~3 times as resin volume. The remaining NaCl was washed clean with water. Because 732 cation exchange resin should be converted to hydrogen strong acid resin, it was treated for 2 h above with 7% HCl solution which was 6 times

as the volume of resin; Similarly, 711 anion exchange resin was treated for more than 2 h with 8% NaOH solution.

Concentration and drying method: Soybean polypeptide fluid was treated by using rotary evaporator for vacuum concentration. Low temperature and rapid speed was expected in concentration process. Concentration temperature was 58~60°C, vacuum degree was 0.09 mPa. Solids content of soybean polypeptide was 35~40% when it entered into the spray drying tower. Inlet temperature and outlet temperature of the spray drying tower were, respectively 135~140°C and 70~75°C.

RESULTS AND DISCUSSION

Influence of flavourzyme dosage on soybean polypeptide bitterness: In the soybean protein Alcalase hydrolysate, the ratio of enzyme and substrate was, respectively adjusted as 10, 15, 20, 25 LAPU/g. And then the soybean protein Alcalase hydrolysate was further hydrolyzed for 2 h by adding Flavourzyme enzyme with constant hydrolysis temperature 50°C and pH7.0. Supernatant fluid that was taken after centrifugal separation to Flavourzyme hydrolysate was tasted. From this process debitterizing effect of Flavourzyme enzyme was determined. The experiment result was showed in Fig. 1.

It can be seen from Fig.1 that hydrolysate bitterness value was smaller and smaller with the extension of reaction time, when reaction was more than 2 h bitterness value changed slowly. When the concentration ratio of enzyme and substrate (E/S) increases, the bitterness value of Flavourzyme hydrolysate reduced quickly. When E/S was 20 LAPU/g and 25 LAPU/g and reaction time was 2 h, bitter taste value was minimum value and when hydrolysis time was extended, bitter taste value no longer reduced. Therefore, it was believed that good debitterizing effect can be achieved when concentration ratio of enzyme and substrate (E/S) was 20 LAPU/g with reaction time 2 h.

Influence of Flavourzyme on hydrolysis reaction:

The pH of Flavourzyme hydrolysate had no obvious change hydrolysis when reaction time was extended from 0 to 3 h with adding Flavourzyme into Alcalase Hydrolysate. Degree of Hydrolysis (DH) and protein recovery of soybean separation protein hydrolysate after Flavourzyme hydrolysis was measured as the results of Flavourzyme hydrolysis. It was showed that the DH of hydrolysate had obvious rising trend with the extension of hydrolysis time, on the other hand protein recovery rate had no obvious rising trend. The experimental results were showed in Fig. 2 and 3.

It can be seen from Fig. 2 that the DH of hydrolysate increased with the extension of hydrolysis

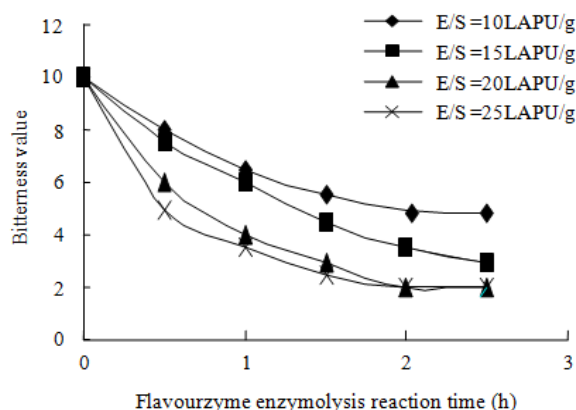


Fig. 1: Affect of Flavourzyme dosage on hydrolysate bitterness

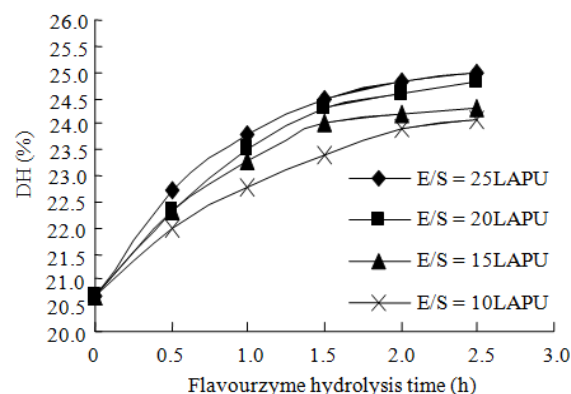


Fig. 2: Affect of Flavourzyme on degree of hydrolysis

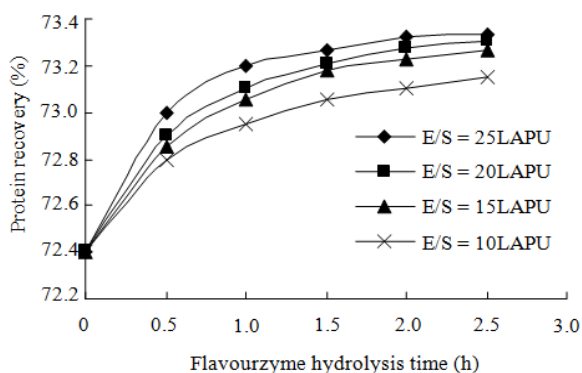


Fig. 3: Affect of Flavourzyme on protein recovery

time. It was because that the peptide bond could be constantly cut by Flavourzyme, when hydrolysis time was extended the number of peptide bond that was cut increased. It can be seen from Fig.3 that protein recovery had no obvious when hydrolysis time was more than 1 h. However, at the same time DH was still increasing. So, it was deduced that Flavourzyme mainly had effect on the peptides which was already hydrolyzed by Alcalase.

It can be seen from Fig. 2 and 3 that protein hydrolysate with lower bitterness value can be achieved

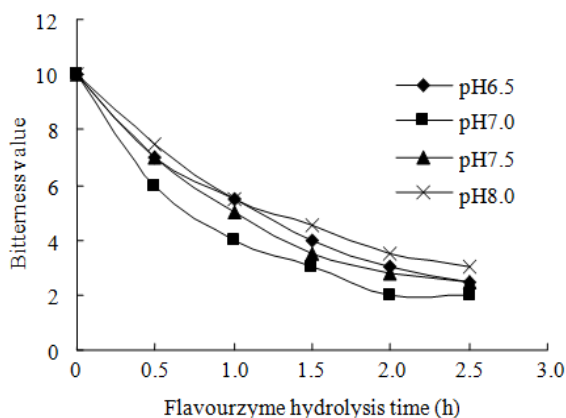


Fig. 4: Affect of pH on Flavourzyme Hydrolysate bitterness

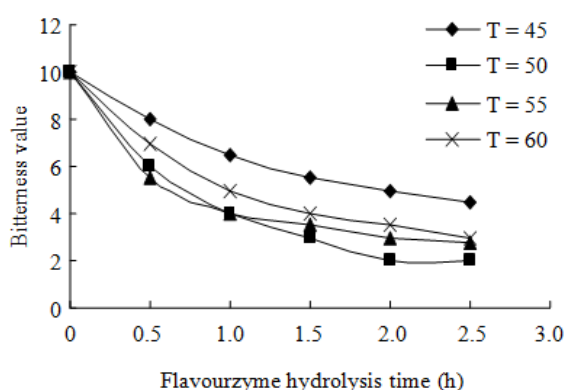


Fig. 5: Affect of temperature on Flavourzyme Hydrolysate bitterness

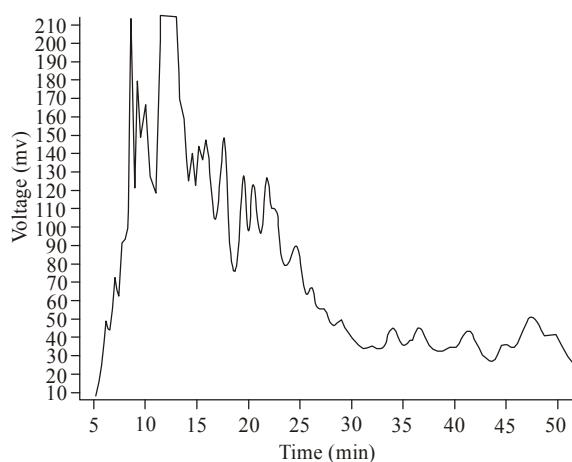


Fig. 6: HPLC diagram of soybean peptides Flavourzyme hydrolysate

after 2 h reaction time with adding 20 LAPU/g Flavourzyme when pH and temperature was 7.0 and 50°C, respectively.

Influence of pH on soybean Flavourzyme hydrolysate bitterness: The pH of Alcalase

hydrolysate was adjusted as 6.5, 7.0, 7.5 and 8.0, respectively. Then Flavourzyme was added with E/S at 20 LAPU/g and 50°C reaction temperature. Bitterness value of Flavourzyme hydrolysate at different pH was shown in Fig. 4.

It can be seen from Fig. 4 that the influence of pH on debittering effect of Flavourzyme Hydrolysate was not large. Enzyme activity of Flavourzyme was strongest because hydrolysate bitterness was smallest when pH was 7.0. So pH 7.0 was determined as optimal enzymatic hydrolysis condition.

Influence of temperature on flavourzyme hydrolysate bitterness: Enzyme hydrolysis was also affected by hydrolysis temperature. The optimum temperature must be studied to maximize the effect of enzymes. When pH of Alcalase hydrolysate was adjusted at 7.0, E/S was 20 LAPU/g, hydrolysis temperature was adjusted at 45°C, 50°C, 55°C and 60°C to find optimum temperature. Bitterness value of Flavourzyme hydrolysate at different temperature was shown in Fig. 5.

It can be seen from Fig. 5 that when temperature was 40°C, 55°C and 60°C bitterness value was bigger than it at 50°C. That is to say when temperature was 50°C Flavourzyme hydrolysate bitterness was smallest with reaction time 2 h. So 50°C was determined as optimal enzymatic hydrolysis temperature.

Molecular weight distribution of Flavourzyme hydrolysate: Molecular Weight Distribution of soybean polypeptide Flavourzyme Hydrolysate was measured by adopting HPLC (High Performance Liquid Chromatography) method which chromatography operating conditions was that chromatographic column was phenyl column 4.6*150 mm, mobile phase was phosphate buffer solution-acetonitrile 90:10 (pH7.0) which concentration was 0.02 mol/L, detection wavelength was 254 nm, velocity was 1 mL/min, column temperature was 25°C. Experimental result was shown in Fig. 6.

It can be seen from Fig. 6 that the change of molecular weight distribution range of soybean protein hydrolysate which was prepared from further Flavourzyme hydrolysis was not big. The change of molecular weight distribution range from Alcalase hydrolysate to Flavourzyme hydrolysate was not obvious.

Affect of activated carbon dosage on decolorizing effect to Flavourzyme hydrolysate: When the dosage of activated carbon powder was respectively 0.5, 1.0, 1.5, 2.0% of the soybean polypeptide Flavourzyme hydrolysate, stirring adsorption was carried on for 1 hour at 50°C. Various solutions which dosage was different were compared, the result was listed in Table 1.

It can be seen from Table 1 that the decolorizing effect of soybean peptide solution was more obvious with the increase of activated carbon

Table 1: Decolor effect by active carbon on soybean polypeptides

Activated carbon dosage % (w/w)	0	0.5	1.0	1.5	2.0
Soybean polypeptide solution (mL)	500	500	500	500	500
Total solution after adsorption (mL)	500	520	540	565	585
Protein content (g/100 mL)	6.08	5.73	5.40	4.90	4.45
Protein loss (%)	0	2.0	4.1	8.9	14.4
Color	Brown	Yellow	Buff	Micro yellow	Micro yellow

Table 2: Ion exchange desalination effect on soybean polypeptides

Soybean polypeptides solution	Solution quantity (mL)	Protein content (g/100 mL)	Conductivity ($\mu\text{S}/\text{cm}$)	Ash content (dry basis)
Entrance	1000	5.37	$>1 \times 10^4$	14.65%
Exit	1080	4.85	1.84×10^3	2.11%

dosage. When activated carbon dosage achieved 1.5% of Flavourzyme hydrolysate, soybean peptide solution had a good color as the color when the dosage of activated carbon was 2.0%. However, activated carbon dosage was more, the adsorption amount of protein was also more resulting in a large number of protein loss bring the economic benefit loss. So the dosage of activated carbon should be reduced in order to reduce the protein loss.

The dosage of activated carbon was adjusted as 1.1, 1.2, 1.3 and 1.4% of solution. Then soybean peptide solutions were made adsorption decolorization processing. Their decolorization effect was compared to the effect when activated carbon dosage was 1.5%. It was found from experiment result that when the activated carbon dosage of solution amount was 1.2% good decolorizing effect could be achieved, at the same time the color of solution was light yellow. The protein loss rate in solution when the activated carbon dosage was 1.2 was 5.7% which was much less than 8.9% when the dosage was 1.5%.

Desalination effect on soybean polypeptides of ion exchange: In the desalting process by using anion and cation exchange resin column, desalted fluid must flow through firstly cation exchange resin column, then it flow through anion exchange resin column. Because if desalted fluid which may contain Ca^{2+} or other cation flow through firstly anion exchange column which may contain OH^- , Ca^{2+} or other cation and OH^- ions could combine to generate precipitation which can jam resin, the column efficiency was reduced or exchange capacity was lost.

Treated fluid after flowing through cationic exchange column was strongly acidic due to the H^+ from cationic resin. When the pH value of effluent solution and the influent solution was roughly the same, the ion exchange was saturated. Similarly, because the OH^- from anion exchange resin into the solution, the pH value of effluent solution increases, when the pH value of effluent solution and the influent solution was roughly the same, the anion exchange resin was also saturated.

Both loading quantity of anion column and cation column were 300 mL. The soybean peptide solution after 1 h activated carbon decolorizing flowed into 732

cation exchange column. The pH of effluent solution was measured which was compared with the value of influent solution. When sample quantity was 7.5 times as column volume, cation exchange resin was saturated. When sample quantity was 5 times as column volume, 711 anion exchange resin was saturated.

It can be decided that the suitable loading column quantity ratio of anion resin and cation resin was 2:3(5:7.5). Loading column quantity of 732 cation exchange resin column was 200 mL. The capacity of 711 anion exchange resin column was 300 mL. 1000 mL soybean protein solution after decolorizing was made desalination processing in resin column. Finally soybean peptide solution residual in column was eluted with no salt water. The result was listed in Table 2.

It was can be seen from Table 2 that protein lost was 2.46% [counting equation was $(5.37 \times 1000 - 4.85 \times 1080) / 5.37 \times 1000$] which loss rate is not high after ion exchange desalination processing. Conductivity of solution reduced largely after desalination processing, because ion in solution was removed by ion exchange resin, ion concentration decreased. It can be concluded that ideal desalting effect was achieved. From the ash content of entrance solution and exit solution, it can be seen that 86% salt was removed by anion and cation exchange resin which effect was also good.

Soybean polypeptide product analysis: Soybean peptide solid powder was gained from concentration drying of soybean peptide solution. The color of peptide powder was milk white or light yellow. The powder was soluble in water and there was no precipitation after dissolving. The taste of 1% aqueous solution of soybean peptide solid powder was light and there was no peculiar smell or bitter. Water content of final product was 5% and protein content was up to 97% (dry basis). Soybean peptide solid powder was easy soluble in acid which soluble Nitrogen Index (NSI) was 99.7%.

CONCLUSION

Soybean protein Alcalase hydrolysate was further hydrolyzed by adopting Flavourzyme as hydrolytic enzyme to reduce bitterness of polypeptide. Bitterness

of soybean polypeptide was reduced distinctly after this further hydrolysis reaction. The optimal hydrolysis conditions of Flavourzyme in Alcalase hydrolysate was determined as that pH was 7.0 at temperature 50°C and E/S(ratio of enzyme and substrate) was 20LAPU/g. Bitterness value was reduced to 2 after Flavourzyme hydrolysis reaction for 2 h in optimal hydrolysis conditions.

It can also be concluded From the HPLC diagram of soybean peptides Flavourzyme hydrolysate that the change of molecular weight distribution range from Alcalase hydrolysate to Flavourzyme hydrolysate was not obvious. Molecular weight of Flavourzyme hydrolysate also focused on the range of small molecules. DH of soybean protein hydrolysate was increased to 24.2% which was improved 3.5% than Alcalase hydrolysate. Protein recovery proportion was increased to 73.2% which was improved 0.8% than Alcalase hydrolysate.

Soybean polypeptide Flavourzyme hydrolysate was decolorized by adopting activated carbon adsorption process. Anion and cation exchange process was applied in the desalination processing of soybean polypeptide. Ideal effect of decolorizing and desalination was achieved.

Optimal dosage of activated carbon was 1.2% hydrolysate (w/w). Soybean polypeptide solution color was light yellow after decolorizing. Ratio of anion resin and cation resin of ion exchange resin was 2:3(V/V). The volume of hydrolysate processed was 5 times as the volume of anion exchange resin. After desalination processing ash content of soybean peptide solution reduced to 2.11% (dry basis), salinity decreased by 86%, on the other hand taste of soybean polypeptide was lite.

After vacuum concentration and spray drying, the final product that was milk white or yellowish solid was gained from soybean peptide solution. Water content of

final product was 5% and protein content was 97% (dry basis). The product powder was easy soluble in acid which soluble nitrogen index (NSI) was 99.7%.

ACKNOWLEDGMENT

This study was supported by spark plan project in Shandong province (No.2011XH06006) and Science and technology development project in Weifang city (No.20121333).

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