

Performance Enhancing Soy Milk Extraction By Flavourzyme

Nguyen ThiQuynhHoa, Nguyen Phuoc Minh, Dong ThiAnh Dao

Abstract-This paper studies the hydrolysis performance of soybean protein by enzyme Flavourzyme aims to produce soy milk products with high protein content. Experiments conducted survey of nutrition of soybeans as moisture content (12.35%), protein (38.91%), lipid content (14.60%), check the enzyme activity (222.638 U/g), survey of the factors affecting the process of hydrolysis as the substrate: water, enzyme activity, pH, temperature, time of hydrolysis process to soluble protein recovery performance. Results are as follows: the best substrate ratio:water is 1: 6, enzyme activity is 27.828 U/g raw beans, pH is 6.5 and appropriate temperature 50°C in 120 minutes. When it dissolves, soluble protein recovery performance reached 52.571%.

Keywords:enzyme hydrolysis, soybean protein, enzyme activity, flavourzyme, soy milk, soluble protein recovery, high protein.

1 INTRODUCTION

Soybeans are known as *Glycine Max* (L.) Merrill of the Fabaceae, *Glycine*, *G.max* species originating from East Asia, widely cultivated throughout the world. With the evidence of language, geography, history shows soy is known about XI century BC in Eastern North China. From the first century to XV-XVI, soybeans strong common to other countries such as Japan, Indonesia, the Philippines, Vietnam, Thailand, Malaysia, Burma, Nepal and Northern India. However in the last decade, 1940, 1950 and till now, America has crossed China and the Orient in the soy industry [11]. Although there are many different foods from soy but the six most important foods from soy are miso, soy sauce, tempeh, tofu, soy milk and oil. Also beans and bean sprouts also carry a high nutritional value [12].

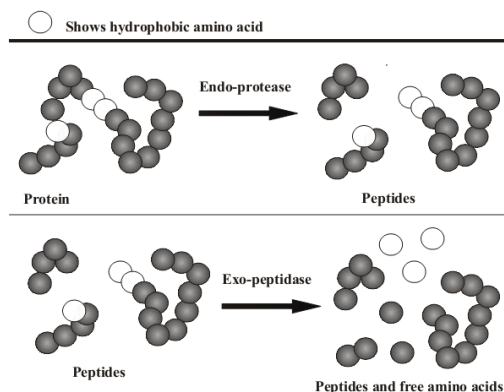


Figure 1. Method of hydrolysis of endo-proteases and exo-peptidase [13]

Soy milk is a product with water extracts of soya beans. Soy milk is an emulsion or suspension, including water-soluble proteins, carbohydrates and fats. Flavourzyme is produced by fermentation of the fungus *Aspergillus oryzae*, used more in the hydrolysis of plant and animal protein. Flavourzyme is proteolytic enzymes such as proteases, peptidase in terms of pH-neutral or slightly acid. Flavourzyme hydrolyzed protein can be of different types and can be used to reduce the bitterness of the following protein hydrolysis when low hydrolysis degree. Flavourzyme enzyme hydrolysis peptide sequences into protein, peptide sequences into the amino acid or peptide sequences are shorter increases amino radicals. Flavourzyme includes endo-proteases and exo-peptidase so Flavourzyme is nature of this enzyme. Endoprotease cut random peptide bonds from the peptide chain is shorter. Exopeptidase can cut the peptide bonds at the beginning of N (aminopeptidase) or at the top of C (carboxypeptidase) from the polypeptide chain is shorter and amino acids. Exopeptidase by Flavourzyme includes large quantities of aminopeptidase and carboxypeptidase [13]. Many studies have shown the benefits of using soy products as prevent cardiovascular diseases, blood cholesterol, cancer, kidney disease, high blood pressure, ... Soy milk product that can meet the demands on extraction process, however the components of soybean by high-performance countries, does not take a rich source of protein and soy beans in the balanced. Therefore hydrolysis method was proposed to solve the problem. There are many methods to soy protein hydrolysis as hydrolysis by acids or hydrolysis by enzymes. However the method of hydrolysis by enzymes bring many benefits were superior as carried out in temperate conditions should create fewer impurities, no reverse reaction and decomposition reactions like hydrolysis method by acid-effective in terms of nutrition, the reaction can proceed in conditions of high substrate concentration which reduces the cost-effects result economically. Such a method of hydrolysis by the enzyme of choice to resolve the issues rose. Derived from the actual demand on the subject "performance enhancing soy milk extraction by the method of hydrolysis by enzymes Flavourzyme" was implemented to provide more information on the factors affecting the process of hydrolysis and the effectiveness of the process, from which base opens new research for soy milk product lines.

- Nguyen ThiQuynhHoa, Cho Ray Hospital, Vietnam; Email: mountainandflower@yahoo.com
- Dong ThiAnh Dao; Vietnam National Uni. HCMC University of Technology, Vietnam; Email: dtanhdao@hcmut.edu.vn
- Nguyen Phuoc Minh; Tra Vinh University, Vietnam; Email: dr.nguyenphuocminh@gmail.com

2 MATERIAL AND METHODS

2.1 Raw material

2.1.1 Soybean

The soybean used in this study is that soy planting in Dong Nai province (Vietnam), soybean is purchased at Long Tan Phu co., Ltd (Ho Chi Minh City).

2.1.2 Enzyme Flavourzyme

Commercial enzyme preparations used in the study are enzymes Novozymes company Flavourzyme® 500 mg (Denmark). The Enzyme was acquired in the company Breen Tag Vietnam (202 Hoang Van Thu Street, Ward 9, Phu Nhuan District, HCM City, Vietnam).

2.2 Research method

2.2.1 Research objectives

Provide information on the factors affecting the process of hydrolysis of soybean and the advantages of the method of hydrolysis by enzymes Flavourzyme compared with water extraction method to performance recovery of soluble protein of molecular weight and to improve the nutritional value of soy milk. Specific impact survey of the factors affecting the process of hydrolysis as the rate base: water, enzyme activity, pH, temperature, time. Through the method of optimization we choose the enzyme activity, pH, temperature, and time to reach the soluble protein recovery performance. Comparison between enzyme hydrolysis method by Flavourzyme and water extraction method of performance recovered soluble protein, molecular mass.

2.2.2 Demonstration research scheme

Evaluate the quality of raw materials Soybeans are checking moisture content by the method of drying to constant weight, total protein test according to the Kjeldahl method, check the total lipid content by the method Soxhlet and enzyme activity is determined Flavourzyme. Survey of the process of hydrolysis of protein from soybeans Determine the effect of the ratio of organic substances: water, enzyme activity Flavourzyme hydrolyzed conditions, such as pH, temperature, processing time enzymes to dissolve the protein retrieval performance. Prepare materials: soybeans are soaked in water at 40°C to bean ratio is 1: 5 during 4 hours in order to allow for the soy seeds Zhang blooms, easy to peel, the recovery performance compounds in soybeans higher [14]. Soybeans will be grinding with water at ambient temperature. Then the mixture is correct pH, addition of enzyme activity with Flavourzyme. Templates are put in hot climate control has a shake with temperature and processing time depending on the experiments. After the processing time enzymes determine the sample will be infinitely enzyme activity at a temperature 90°C during 10 minutes and vacuum filtration was conducted to collect room hydrolysis. Translate hydrolysis is conducting performance tests soluble protein recovery. All experiments were repeated three times to obtain the average value between 3 repetitions. The average value was considered significant difference when $P < 0.05$. The results were statistically processed by software Statgraphics Centurion XV.I.

2.3 Analytical methods

2.3.1 Moisture content

Guidelines: sample drying to constant weight analysis to infer the amount of water present in the original sample.

2.3.2 Concentration of total protein

Total protein is determined by determining the total N content according to the Kjeldahl method (AOAC 984.13, 2000) multiplied by 5.47. Principle: heat model offers analysis with concentrated H_2SO_4 , the oxidized organic compounds. Carbon and hydrogen to form CO_2 and H_2O , whereas nitrogen after it is emitted in the form of NH_3 will combine with $(NH_4)_2SO_4$ form H_2SO_4 to dissolve in the solution. Chase out NH_3 $(NH_4)_2SO_4$ by NaOH and store the excess amounts of NH_3 and H_3BO_3 with 3%. Titrated with H_2SO_4 0, 1N standard will determine the amount of NH_3 was born.

2.3.3 Concentration of total lipid

Lipid content is determined according to Soxhlet method (AOAC 960.39, 2000). The principle: Use solvents the water horse (diethyl ether, petroleum ether) lipid extraction complete soy ingredients are finely ground. Some fat soluble component extracted also glass by including pigments, fat soluble vitamins, substances that smell. Due to the impurities, the extraction is called total lipid or crude oil. Then completely evaporated the solvent, weight mass remaining samples and counted out the lipid concentrations in samples of soy flour.

2.3.4 Enzyme activity

Enzyme activity is determined by the method of Anson. This method is based on the hydrolysis of the compound protein (casein or hemoglobin) by the enzyme. Then inactivate enzymes and protein hydrolysis have not been precipitated by TCA (acid trichloroacetic). Product weight is made up of response by color reaction with Folin, result analysis based on graph standard tyrosine. Anson unit definition: one unit of energy is the minimum enzyme Anson in experimental conditions (35.5°C; pH 7.5) hydrolysis of casein in 1 minute forming soluble products of TCA, react with Folin which represents absorption OD at 660nm corresponds to 1 μ mol Tyrosine in Dateline [3].

2.3.5 Concentration of soluble protein

Soluble protein content was determined by the method of Lowry. Principle: Folin-Ciocalteu containing phosphomolipidic acid and phovolframic acid. This method relies on the Biuret reaction, in which the peptide bonds of proteins react with Cu in alkaline environment to create the Cu^+ ion, Cu^+ creates will react with Folin-Ciocalteu created purple blue solution. Color intensity depends on the composition of protein tyrosine and tryptophan amino acid radicals in this by participating in the process of creating colored complexes [2, 7]. First standard road construction with a pure protein is usually the albumin protein concentrations to determine the melt in model experiments. From the rear, when the optical density value will identify the protein concentration. The optical density values are measured with absorbance spectroscopy UV-VIS at wavelength $\lambda = 750nm$. This method is best used with

aqueous protein concentrations ranging from 0.01 to 1 mg/mL.

2.3.6 The degree of hydrolysis of DH

The degree of hydrolysis (DH) is used to measure the ability of a protein protease hydrolysis. The degree of hydrolysis (DH) is the percentage of the peptide bonds was cut in the process of hydrolysis, which is the key parameters describing product characteristics proteolytic [6]. $DH (\%) = (\text{number of peptide bonds cut} / \text{total}) \times 100\%$. Principle: determining the degree of hydrolysis (DH) by pH-Stat is done by maintaining the hydrolysis patterns at a constant pH value after a certain hydrolysis with NaOH 0.1N.

2.3.7 The molecular weight

The molecular weight is determined by the method of electrophoresis SDS-PAGE. Principle: SDS-PAGE electrophoresis is a technique on the gel polyacrylamide with SDS is denaturely and negatively molecules, this method determines the protein molecular weight, composition and protein preparations cleanliness during refining. In this technique the protein is processed with SDS detergent and reducing agent for bridge disulfid as mercap to ethanol or DDT do structure 2, 3, 4 of the protein being transformed into tier 1 and negatively. So when the electrophoresis, protein molecules depends only on the size when moving in gel: the large size molecules will move slower than small molecules.

2.4 Method of calculation and data processing

All experiments were repeated three times and the data of experiments conducted computer error and analysis of variance ANOVA a factor (one-way ANOVA) to determine the difference of the data with the meaning and the standard error of $P < 0.05$ software Statgraphics Centurion XV.I aimed to test the reliability of the results obtained from these experiments. The result is expressed in the form: the mean \pm standard deviation.

3 RESULTS AND DISCUSSION

3.1 Results identify the soy ingredients

Raw materials are analysed to determine the nutrient content and results are presented in the table.

Table 1. Soy ingredients per 100 g nuts

Component	Concentration (%)
Moisture	12.35
Total lipid	14.60
Total protein	38.91

Particles have low relative moisture content can be preserved without being germinated during the study. The results show high protein soy occupies 38.91% in line with the selection criteria of material made in research. At the same time the result is also consistent with the statement of the composition of soy nuts [5]. According to w. f. Wilkens

(1969) [14], moisture in the soy is 12-13%, the moisture content of the soy beans in the study fit with this result. By NacerBellaloui (2010) [9], protein in the range 34.1-56.8%, mean value of about 42.1% of the mass of particles; lipid concentrations in the range of 8.3-27.9%, worth an average of 19.1% of the mass of particles. The result analysis of proteins and lipids between upper and lower value average value. According to Ajay k. Dixit (2011) [4], a protein of about 38% lower than the result analysis of lipid content in the table, about 20% higher value analysis. Such a protein, lipids of soybean seeds with proper scientific research. The result is higher or lower than the analysis of the equivalent value of the authors on this may be due to the difference in varieties of soybeans, edaphic, climatic conditions, conditions of care.

3.2 Results determine the enzyme activity of Flavourzyme

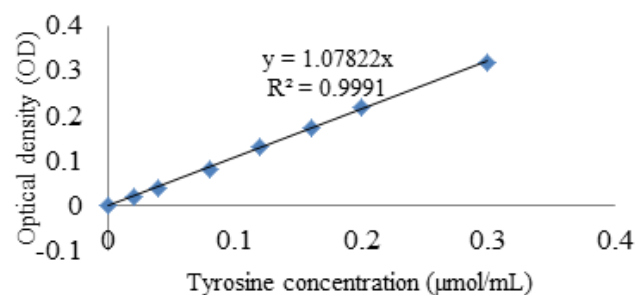


Figure 2. Tyrosine calibration curve

In the process of preserving the enzyme activity will decrease over time, but in the process of doing experiments, this change is not significant, does not affect the results of experiments. So to avoid reducing enzyme activity, the enzyme needs to have reasonable preservation mode as preserved at a temperature of 0-10°C, the enzyme splits the smaller volume, avoid light shining directly or exposed to air often.

Table 2. Results determine the enzyme activity

Experiment	OD	OD blank	ΔOD	x (µmol/mL)	Activity (UI/g)	Average activity (UI/g)
Time 1	0.1719	0.0216	0.1503	0.13940	223.0336	222.638 \pm 0.453
Time 2	0.1713	0.0216	0.1497	0.13884	222.1433	
Time 3	0.1717	0.0216	0.1501	0.13921	222.7368	

3.3 Survey of the process of hydrolysis of protein from soybeans

3.3.1 Effect of substrate: water ratio to protein hydrolysis

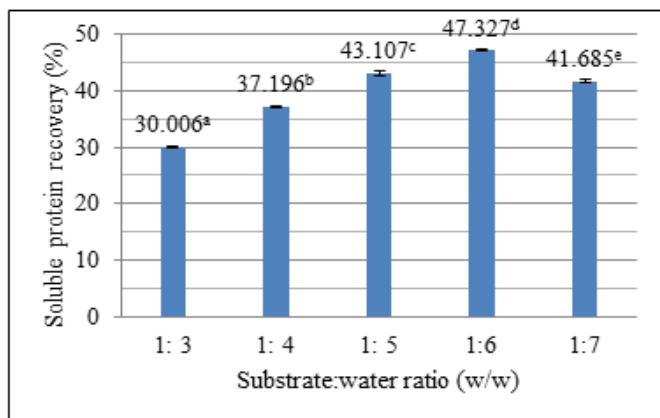


Figure 3. Effect of substrate:water ratio (w/w) to the soluble protein recovery efficiency (%)

When increasing the concentration of organic substances from water: body ratio is 1: 7 to 1: 6 then the recovery performance soluble protein increased from 41.685% to 47.327%, but when it continues to increase the concentration of the substance from a ratio of 1: 6 to 1: 3 then the recovery performance protein does not increase that fell from 47.327% to 30.006% down; According to the analysis of Anova and LSD, in the ratio of 1: 3, 1: 4, 1: 5, 1: 6, 1: 7 then the recovery performance of dissolved proteins have significant differences ($p < 0.05$) statistically at the 95% confidence level. Physical body proportions at 1: 6 for performance the highest soluble protein recovery rate 1: 6 was used for subsequent experiments. As for the hydrolysis reactions by enzymes, water is not the environment to diffuse muscle enzymes and substrates but also is involved in reactions; affect the speed and the direction of the hydrolysis reaction. So the ratio of co compounds: water affects soluble protein recovery performance [2]. When the concentration of organic substances that increase IE when the rate base: water increased from 1: 7 to 1: 6 then the reaction rate increases, the likelihood of contact between enzymes and organic matter increases, so the soluble protein recovery performance also increases as in the graph [1]. When the physical body: water rate continued to increase from 1: 6 to 1: 3 then the soluble protein recovery performance decrease is due to the concentration of high-quality body viscous mixture makes the enzyme hard diffuser and come into contact with the substance, which reduces the speed of the reaction. At the same time less water should be able to entice the protein from soybean seeds in the fall, since it hinders the ability to catalyze the hydrolysis of muscle enzymes that cause soluble protein recovery performance is reduced. At the same time the concentration of organic substance is too high also inhibited enzyme activity due to multiple molecules organic substances could be linked to the enzyme molecules, these complexes cannot be converted to create products should decrease enzyme activity [1].

3.3.2 Effect of enzyme activity to the process of hydrolysis of proteins

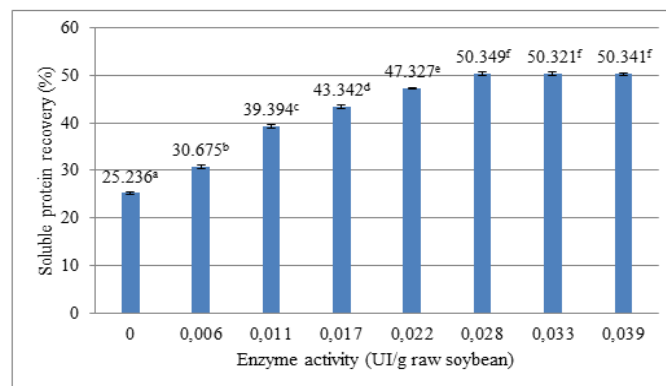


Figure 4. Effect of enzyme activity (UI/g raw soybean) to soluble protein recovery (%)

When activated the enzyme increases from 0 to 27.828 UI/g beans, raw material recovery performance soluble protein increased from 25.236% to 50.349%, but when counting enzyme activity from 27.828 UI/g raw beans to 38.960 UI/g beans, raw material recovery performance soluble protein does not increase that reached equilibrium. According to the analysis of Anova and LSD, enzyme activity at 0 UI/g raw beans; 5.566 UI/g raw beans; 11.130 UI/g raw beans; 16.696 UI/g raw beans; 22.262 UI/g raw beans; 27.828 UI/g raw beans, soluble protein recovery performance difference is significant and enzyme activity in 27.828 UI/g raw beans; 33.394 UI/g raw beans; 38,960 UI/g beans, raw material recovery performance soluble protein did not differ statistically significant at the 95% confidence level. Enzyme activity in 27.828 UI/g raw beans for protein recovery performance soluble enzyme activity should peak at 27.828 UI/g beans selected materials used for subsequent experiments. With the same concentration of organic substance, when increasing the amount of the enzyme used is in the mix will have more exposure and enzyme hydrolysis of organic substance, so the products created even more. That explains when to use enzyme activation from 0 UI/g raw beans to 27.828 UI/g raw beans, increasing protein recovery performance. But once the substance has been linked before with enzymes to metabolize the products, the reaction does not change or did not rise further when increased enzyme activity [13]. That explains why when increase enzyme activity from 27.828 UI/g raw beans to 38.960 UI/g beans, raw material recovery performance soluble protein did not have meaningful differences. In addition use many enzyme so inefficient in terms of performance recovered soluble protein and also increases the costs for the production process. When comparing samples of confronting or enzyme activity by 0 UI/g raw beans for the hydrolysis of the hydrolysis patterns are soluble protein recovery performance greater than sample confronting with the differences mean. Specifically in the enzyme activity by 27.828 UI/g raw beans, soluble protein recovery performance reached 50.349% twice each confronting. It shows the effectiveness of the methods of hydrolysis of soluble protein recovery performance.

3.3.3 Effect of pH medium hydrolysis to protein hydrolysis process

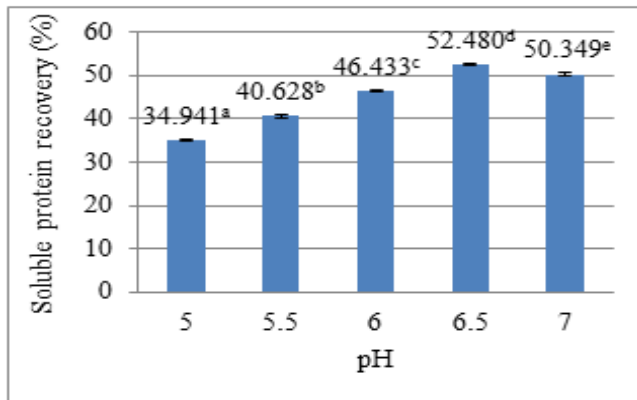


Figure 5. Effect of pH medium to soluble protein recovery (%)

When pH increases from 5 to 6.5, the soluble protein recovery performance increases from 34.941% to 52.480%, but when the pH increased from 6.5 to 7 then the recovery performance soluble protein does not increase that fell from 50.349% to 52.480%. According to the analysis of Anova and LSD, pH at 5; 5.5; 6; 6.5; 7 the soluble protein recovery performance difference statistically significant at the 95% confidence level. pH at 6.5 for performance the highest soluble protein recovery should the pH 6.5 was used for subsequent experiments. pH affects the hydrolysis reaction is caused by the process of ionization or muscle enzymes. This process can form the link causes the substance becomes tight and difficult than hydrolysis. In addition the process of ionization can also do product change and affect the durability of the enzyme. This process also affects amino acid carboxyl groups by influencing and amine, to change the spatial structure of proteins and affect the ability of enzyme activity [13]. Thus each enzyme has a pH optimum between pH and higher or lower than the change in the value of optimization is the process of hydrolysis is affected.

3.3.4 Effect of medium temperature hydrolysis to protein hydrolysis process

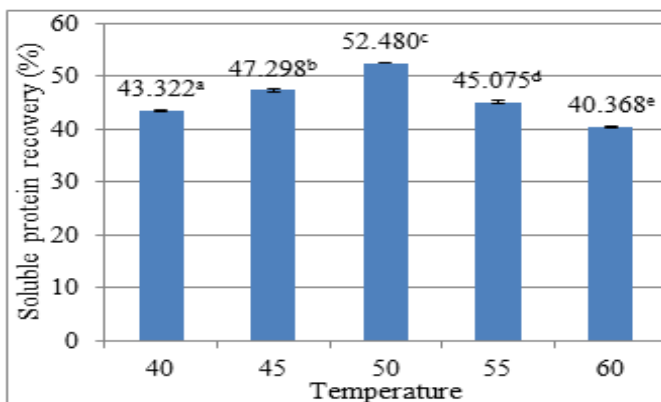


Figure 6. Effect of temperature to soluble protein recovery (%)

When the temperature increases from 40°C to 50°C the soluble protein recovery performance increased from 43.322% to 52.480%, but when it continued to increase in temperature from 50°C to 60°C the soluble protein recovery performance is not increased but decreased from 52.480% to 40.368%. According to the analysis of Anova and LSD, the temperature at 40°C; 45°C; 50°C; 55°C 60°C, then the recovery performance soluble protein difference statistically significant at the 95% confidence level. The temperature at 50 °c for performance the highest soluble protein recovery should the temperature selected 50 used for subsequent experiments. As the temperature increases the kinetic energy of the molecules increases, the molecules that are the typical muscle enzymes and substrates easy movement and contact with each other. So when the temperature increases in a certain period, the enzyme activity increased and the speed of response increases. That explains when the temperature increases from 45°C to 50°C, then increasing protein recovery performance. When the temperature rises beyond a certain value, the protein can be denatured. Enzymes are protein enzyme so when nature is being lead to the enzyme activity center with no longer conform to the substance. That explains why when the temperature increases from 50°C to 60°C the soluble protein recovery performance is reduced.

3.3.5 Effect of enzyme treatment duration to protein hydrolysis

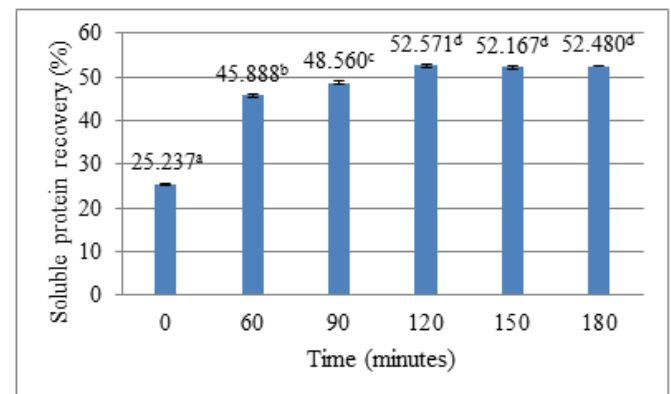


Figure 7. Effect of enzyme treatment duration to protein hydrolysis recovery(%)

When time increases from 0 minutes to 120 minutes then the recovery performance soluble protein increased gradually from 25.237% to 52.571%, but as time continues to increase from 120 minutes to 180 minutes, soluble protein recovery performance does not increase anymore which reaches equilibrium. According to the analysis of Anova and LSD, time at 0 minutes; 60 minutes; 90 minutes; 120 minutes, soluble protein recovery performance difference is significant and when time in 120 minutes; 150 minutes; 180 minutes, the recovery performance soluble protein did not differ statistically significant at the 95% confidence level. So the time in 120 minutes for the performance the highest soluble protein recovery should the time 120 minutes was used for subsequent experiments. The process of hydrolysis enzymes need time to cut off the link. The longer the time, the number of links

cut off as much, explaining that when the rise time from 60 minutes to 120 minutes then the recovery performance soluble protein increased from 45.888% to 52.571%. However the process of hydrolysis occurred rapidly in the early stages, when that has large amounts of peptide links severed easily. This explains why recovery performance soluble protein increased in the period from confronting in time is 0 minutes. Recovery performance specific soluble protein in the 60-minute time 45.888 1.82 times higher performance% recovery of soluble protein in time is 0 minutes. Then the hydrolysis speed can decrease when the peptide bonds are prone to hydrolysis at least gradually. The presence of the peptide chain of short circuit the new born can act as basis effective competitive nature with the molecules not hydrolysis or partial hydrolysis [10]. This interpretation when time increases for some time determining the soluble protein recovery performance with no meaningful difference. When comparing samples of confronting or at the time of 0 minutes with samples of hydrolysis, the recovery performance of soluble protein patterns of hydrolysis is confronting higher. This indicates the effectiveness of the process of hydrolysis of soluble protein recovery performance.

4CONCLUSION

Provide information on the factors affecting the process of hydrolysis of soybean and the advantages of the method of hydrolysis by enzymes Flavourzyme to performance recovery of soluble protein of molecular weight and to improve the nutritional value of soy milk. The conclusions given in the study are as follows: Lam Phuong soya from Long Tan Phu company has stable components such as moisture content (12.35%), lipid content (14.60%), protein (38.91%) are used for hydrolysis of high protein retrieval performance achieved. The processing of raw materials prior to hydrolysis as percentage of legumes: water during soaking is 1: 5, temperature 40°C, time is 4 hours are selected to suit increases recovery performance soluble protein.

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