Improvement Ofprotein Extraction From Soybeanby Non-Thermal And Thermal Treatment

Nguyen ThiQuynhHoa, Nguyen Phuoc Minh, Dong ThiAnh Dao

Abstract: This research focused on researching conditions for protein extraction from raw soybeans by enzymatic method under non-thermal and thermal tsreatment. The aim of this research was to get the highest yield of soluble protein recovery and non-bitter taste in protein hydrolysates. Enzyme – hydrolyzed soy protein was Flavourzyme (endopeptidase and exopeptidase mixture) which not only hydrolyzed to create free amino acids, short peptides, increase soluble protein contents in hydrolysates solution; but also non-bitter taste. The survey was carried out in two cases: unheated and heat-treated soybeans. Heat treatment led to a more effective enzymatic extraction. In details as follows:The unheated soybeans: The highest yield of soluble protein recovery was obtained 52.57 ± 0.27 in hydrolysis conditions: ratio of substrate and water was 1/6 (w/w), ratio of enzyme and substrate (E/S, w/w) was 12.5% (27.75 UI/g), pH was 6.5; 50^{0}C; 120 minutes.Soybeans were heat-treated in pressure cooker with ratio of water and soybean was 8/1 (w/w) for 20 minutes, then hydrolyzed by Flavourzyme with ratio of substrate and water was 1/3, E/S was 7% (15,54 UI/g), pH was 6.5; 50^{0}C; 150 minutes, the highest yield of soluble protein recovery was obtained 61.44 ± 0.22%.

Keywords: soybean, protein extraction, enzymatic method, non-thermal, thermal treatment, soluble protein, Flavourzyme

1 INTRODUCTION

Soybean scientific name is Glycine Max Merill, according to FAO statistics (1994). Soybeans are botanically classified as follows:

Kingdom	: Plantae
Rank	: Magnoliophyta
Class	: Magnoliopsida
Order	: Fabales
Family	: Fabaceae
Subfamily	: Faboideae
Genus	: Glycine
Species	: max

Scientists based on morphology, unify the views that the soybeans originating fromChina, comes from a type of wild soybean torso piece, the form of vines. From Chinese,soy spread all over the world [1, 5]. There is a lot of interest in the study of active peptide.

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These peptides are found fromthe process of hydrolysis of milk proteins, eggs, fish, whole grains, soy (Matsui et al.,1993; Liet al., 2002; Yoshikawa et al., 2003) but in which the hydrolysis products from soybeans is being viewed the most. In soy withmany ingredients have effects against cancer (Kennedy, 1995; Lumen, 2005, Jeonget al., 2003) and majorstreets to find out what this peptide is hydrolyzed byenzymes. Besides the anticancer effects, soybeans are also impact resistant to hypertension (Wu and Ding, 2001; Kodera and Nio, 2002; Kitts and Wiler, 2003),reducing cholesterol (Bakhit et al., 1994) and decrease in plasma triglyceridelevels (Iritani et al., 1996). In summary, the process of hydrolysis of protein by enzymes not only improves the nutritional value and function of soybeans that can eliminate the resistance componentof nutrition (Lahl and Grindstaff, 1989) improves the digestibility of soy protein in the body. Because of these allergenic properties, poorly soluble, hard to digest soy proteinshould be limiting the use of food and drugs. Today, the proteolytic enzyme bymethods not only alter the amino acid content in soy than not hydrolysis, but alsomake up the features, physical properties and better nutritional value. Many studiesshow that during the hydrolysis of the peptide has antioxidant activity. This peptidesuppresses the formation of free radicals inside the body and eliminate the free radicals.Compare with soy protein, the peptide is easily digested and absorbed, less allergenic,promote lipid metabolism in the body, restore myoglobin, lower choslesterol, preventing hypertension, immune booster and fight free radicals. Add to that the antioxidant activity of these peptides also has anti-aging effects, be many applicationsin cosmetics and food products have health benefits [15]. The research was published from soybeans. Long soybeans were many scientists pay attention to not only the nutritional value which is the beneficial effects for health. In 1999, Christina S Venter found soybeansand soy products take effect: lower cholesterol, fight cancer, prevent osteoporosis, limiting obesity. In 2011, Ajay k. Dixit and also study found the components to function, the beneficial effects on health:

-α-linolenic acid: improved cardiovascular health, reduce levels of triglycerides in the blood.

-Isoflavone lecithin: female hormones is that lower cholesterol, prevent breast cancer,cancer of the prostate, improves lipid metabolism in the body.

-Lecithine: improve lipid metabolism in the body, enhances memory

-Lectin: prevent cancer, strengthens the immune system

-Saponins: adjust the mechanism of lipid metabolism, anti-oxidant[2]

In 2011, the Sui Xiaonan, Jiang Lianzhou activity studies on oxidation of soy peptideconcluded that: the SPI to be hydrolysis by protease enzyme 6 different then Alcalaseenzyme is the hydrolysis of the peptide has more antioxidant activity is best. The optimal hydrolysis conditions are: temperature 52.5^{o}C, pH 8.5, hydrolysis time 5 minutes,the concentration of organic substances of 7%, the concentration of the used enzymeis 9000U/g organic substance. After 5 minutes the hydrolysis, examine the antioxidantactivity that prevents the formation of groups of OH-are 36.43%, ROO-46.24% of activity, is the kind of group O2-formed in 317 activity, 74U/g [15].Besides, there are many studies found the valuable bio-peptide from soybean proteinhydrolyzed with effect: resistant to cancer, anti-hypertensive, reducing the amount of cholesterol and triglyceride in blood plasma[4] Then there are some studies on the extraction of protein from soy:In 2001, Fischer and his team concluded that the use of water extraction of proteinfrom soy flour and fat are not thoroughly, yields are typically from 13-67%; If using the enzyme process that yields a lot higher from 89-94%; also if used to supportcarbohydrase hydrolysis does not differ much compared to the case using proteases. In particular they found if using the thermal process increases the moisture found proteinextraction efficiency is much higher, because the heat that denature proteins helpprotease hydrolysis is easier [10].However, using the enzyme hydrolysis of soy protein, the hydrolysis product is oftenbitter. Therefore, Ma Yongsheng and et al. (2013), conducts research of hydrolysiscondition of soybean polypeptide from Flavourzyme were hydrolyzed before byAlcalase then noticed that the bitter taste of hydrolysis was markedly reduced. Optimal conditions of Flavourzyme is determined at pH 7 and 50^{0}C temperatures, enzyme/ratein organic substance is 20LAPU/g. product's bitterness is reduced 2 times afterhydrolysis by Flavourzyme in optimal conditions in 2h. The degree of hydrolysis of soyprotein hydrolysis is 24.2% increased 3.5% compared to using Alcalase hydrolysis.While protein is 73.2% compared to hydrolysis by Alcalase rises 0.8%[16]. Recognize the nutritional value of soy-protein supplies abundant plants, has many beneficial effects for health. But soy protein with poorly soluble nutrient resistance components, causing digestive difficulty should limit use of food and drugs. Today, the process of hydrolysis of protein by enzymes not only improves the nutritional value, improve the ability to digest proteins in the body, but also the biological meaning given. It is a product of the hydrolysis of protein from soy, for the shorter peptides of molecular, circuit volume low. This is the peptide is active. In addition, most of the previous studies were hydrolyzed from soybean product is over FAT. This process must use organic solvents for extraction of fat, then use the method to pursue all organic solvents are also saved in the product. This phase is also expensive in time and effort. Thus, this topic focuses on problem solving and extraction of protein from soy beans, raw material, not fat and enzymes for hydrolysis. Eager to find out the conditions of extraction of protein from soy beans by enzyme effectively, to improve recovery performance of dissolved proteins at the same time meet the requirements of sensory protein hydrolysis product is no bitter taste.

2 MATERIAL AND METHODS

2.1 Raw material

2.1.1Soybean

-Soy is purchased from a limited liability company membership import &exportPhu Long (85/10 Tran Chan, Ward 14, Dist. 5, HCM City, Vietnam), in order to create the stability of nutritional ingredients, the quality of the raw materialsused in the study.

-Source: Lam Phuong, Dong Nai province

2.1.2 Enzyme

- Enzyme protease (Alcalase 2,4L; Flavourzyme 500MG)
- Enzyme cellulase (Celluclast 1,5L)

Enzyme preparation was bought by the Danish NovozymeR, distributed by BrennTagcompany in Vietnam (202 Hoang Van Thu, Ward 9, PhuNhuan district).

2.2 Research method

This study was conducted in laboratory scale, the object is the soy bean derived from Phuong Lam, Dong Nai province, Vietnam.Protein extraction process uses cellulase and protease enzyme preparations of Novozymes, Denmark. The content of research include the:

- Analysis of some of the chemical composition of soybean seeds.
- Survey of the process of extraction of protein from soybean by enzymatic method:

+ Preliminary survey selected protease enzymes fit the proteolytic process fromsoybeans.

+ Survey of the factors affecting the process of hydrolysis of soy protein by Proteases in both two soybean cases do not pass heat treatment and through heat treatment. Then perform optimization of the hydrolysis process to find the best protein extractionconditions.

Soybean after soaking, in turn changing the amount of water and the corresponding heat treatment timeas on to survey influence thermal regimes of soybean to the effectiveness of protease enzyme hydrolysis process. Check the humidity of soya beans after heat treatment. In the end bring beans have heat treatment go grind and hydrolysis by protease. Sample volume per-hydrolysis soybean seeds 5 g's raw materials. Fixed hydrolysis conditions the rate of organic matter, water, enzyme/rate of organic matter, pH, temperature and time as above. After hydrolysis done collecting performance and identifying

outbreaks get revoked soluble protein. The experimentsare conducted is repeated 3 times.

2.3 Analytical methods

- Determination of total protein content by Kjeldahl method
- Determination of total lipid content by Soxhlet method
- Determination of moisture content in drying method constant
- Determination of soluble protein recovery performance method of Lowry
- Define the level of hydrolysis by pH-stat
- Molecular weight determination by electrophoresis method
- Identify indicators peroxyde by titration method
- Active protease enzymes identified by Anson method.
- Determination of enzyme activity celluclast 1, 5 l by Miller
- Sensory evaluation methodof products hydrolyzed soy powder

2.4 Data handling methods and optimized the process of hydrolysis

The experimental progress of the error count and analysis of variance ANOVA to determine the difference of the metrics with varying meaning $P < 0.05$, standard error and software Statgraphics aims to test the reliability of the results obtained fromthese experiments.To determine the results of optimization experiments the influence of these factors on the objective function, we use the method of the surface meets the RSM (ResponseSurface Method) and 5.0 Modde software to process the results. Response surfacemethod is the method effective in maximizing the food processing process. Here is a collection of mathematical modeling techniques and statistics, the association betweenthe processing of data and establishment of regression equations to describe the input parameters to the nature of the product [3].

3 RESULTS AND DISCUSSION

3.1 Results determine the ingredients of soy

Soy nuts are analyzed to determine a number of ingredients, the specific results to be obtained are as follows:

Table 1. Results identify the soy seeds ingredients

Component	In 100 g nuts(%)	Dry matter content(%)
Moisture	12.35	-
Total lipid	14.60	16.66
Total protein	38.91	44.39

Results of analysis showed the soybeans used in the study of protein makes up 44.39% lipid, accounted for 16.66 percent of dry matter content. This results in line with published by Mian n. Riaz (2006): the protein makes up 35-40% lipid, 15-20% of dry substance content [8]. According to the USDA (2004) protein makes up 36.49g/100 g soya granules, lipids amounting to 19.94g/100 g soy seeds [6]. According to Ajay K.Dixit (2011), protein makes up 36%, lipids accounted for 19% of the dry matter content [2]. Soy bean protein concentrations used in this study are quite high and consistent with the selection criteria of material research. However, the results we obtained is higher than previously announced. It is caused by the difference in plant breeding, edaphic, climatic conditions etc. Besides, the material is also high lipid content (14.60 %) should be noted in the process of hydrolysis and maintained since May of the oxidation of fat affect the product quality of soy beans humidity, accounted for12.35% suitable for preservation in temperatures often in the duration of the study.

3.2 Survey results the process of extraction of protein from soybean by enzyme method

3.2.1Survey results selected protease preparations in line with the process of hydrolysis

To choose protease preparations in line with the process of hydrolysis of soy protein,we investigated two preliminary hydrolysis protease enzymes (Alcalase andFlavourzyme) on soybean seed quality was through heat treatment. The amount of enzyme used to be attributed to the same activity is 15.54UI/g (hydrolysis in a certaintime period) respectively in turn with the concentration of Alcalase and Flavourzyme is0.9536% and 7%. The results obtained are as follows:

Table 2. Survey results table protease preparations soy protein hydrolysis

Types of protease	Alcalase	Flavourzyme
Ratio E/S (%)	0.9536	7
pH	7	7
Temperature (^{0}C)	60	50
Time (minutes)	180	180
Soluble protein recovery (%)	69.62 ± 0.003	58.79 ± 0.21

Note: values are expressed as the mean of three repetitions ± standard deviation

The results obtained notice Alcalase soy protein hydrolysis for soluble protein recovery performance (69.62%) was much higher than the Flavourzyme (58.79 percent). However, when the sensory room hydrolysis then noticed Flavourzyme means than Alcalase is creating hydrolysis products do not have the bitter taste. Usually the protein hydrolysis by enzymes often have bitter taste although initial crude protein did. The results obtained are consistent with studies of Seo et al. (2008), when conducting reviews of bitterness (using TDA methodology: Taste dilution analysis) of products hydrolyzed by enzymes (Flavourzyme, Alcalase, Neutrase, Bromelain, Papain, and Protamex) from soy products (SPI) then noticed: when the level of protein hydrolysis increases the bitterness of hydrolysis increases. But contrast the Flavourzyme hydrolyzed increases gradually when the level that produces the peptides cause bitterness. Protein hydrolysis by enzymes often bitter is due to produce the peptide chain containing hydrophobic amino acid. Many studies show that effective Flavourzyme hydrolysis of peptides containing hydrophobic amino acid at position-NH2 group at the end of the circuit, reduce the bitterness of the hydrolysis products [13]. Soy protein is not

the original, very large protein molecular weight, shape, shrink roll confine the hydrophobic groups on the inside. When hydrolysis by enzymes, spherical structure broke down, to reveal the amino acid hydrophobic properties should translate hydrolysis is obtained with bitter taste [16]. Besides the nutritional value, the sensory properties of the product are important. Therefore, the enzyme Flavourzyme selected for soy protein hydrolysis in order to increase extraction efficiency.

3.2.2 Survey results to factors affecting the process of hydrolysis of soybean protein byFlavourzyme in soybean seeds non-thermal processed

3.2.2.1 Substrate concentration (bean/water ratio, w/w)

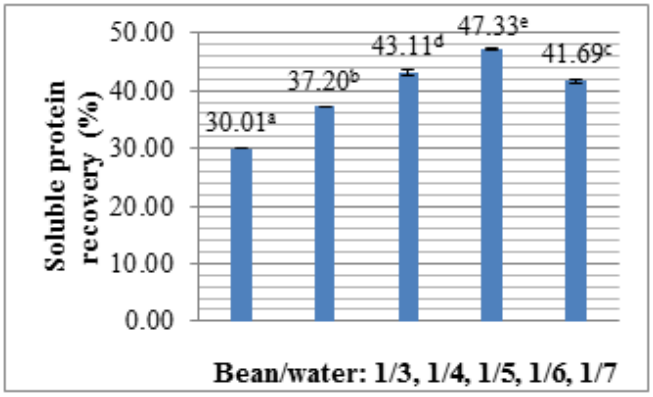

Figure 1. Effect of bean/water to sy protein hydrolysis by Flavourzyme

Note: the values are expressed as the mean of three repetitions ± standard deviation and the same characters (denoted above) then the difference between them is notsignificant (α = 5%).

A number of studies have demonstrated that the rate of organic substances, water may affect the effective process of hydrolysis. In turn changes the ratio of legumes/water from 1/3; 1/4; 1/5; 1/6; 1/7 show me: in passing rate/water from 1/7; 1/6 with respect to the degree of dilution of the reagents decrease means that the concentration of the substance increases the performance increasing soluble protein recovery, specifically from 41.69 percent to 47.33%. Also when in bean rates/water from 1/6; 1/5; 1/4; 1/3 with the degree of dilution of the reagents decrease meant the concentration of organic substance continue ascending the soluble protein recovery performance does not increase that fell, specifically from 47.33 percent to 30.01%. This phenomenon is explained as follows: 1/3, the rate of dilution of the lowest quality, the concentration of the substance is too high so the viscosity of high system enzymes are difficult to disperse and exposure to organic chemicals to cut peptide bonds fit in soluble protein should make up no more means soluble protein recovery performance is not high. Also at the rate of 1/7, the rate of dilution of the highest quality, the concentration of organic substance then the hydrolysis is too low, add to that the enzyme is diluted in water, enzyme hydrolysis exposure is not enough to end the matter. Thus, soluble protein recovery performance is reduced. In summary, only at a rate of organic substances, the appropriate country then translates the hydrolysis of obtained performance achieved recover the highest soluble proteins. Therefore, the need to determine the concentration of the substance is suitable for the reaction. In this survey, we see the process of hydrolysis of protein from soybean by Flavourzyme in absence of heat treatment reaches the highest protein recovery performance (47.33 percent) in the physical body/water ratio is 1/6.

3.2.2.2 Enzyme /substrate(E/S, w/w, %)

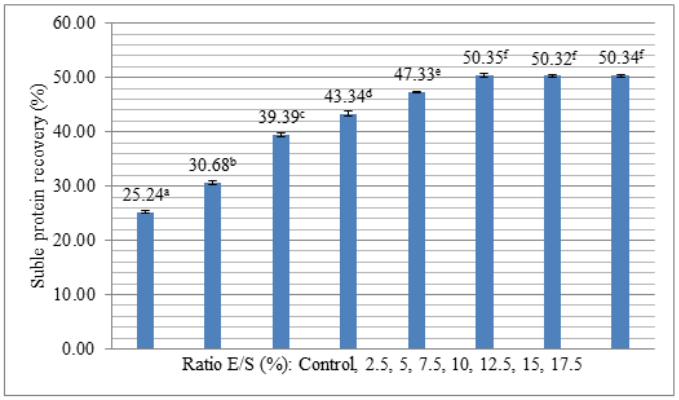

Figure2. Effect of E/S to soy protein hydrolysis by Flavourzyme

Note: the values are expressed as the mean of three repetitions ± standard deviation and the same characters (denoted above) then the difference between them is notsignificant (α = 5%).

Survey of the influence of rate of E/S to the hydrolysis process with the aim of finding suitable uses enzymes that process of hydrolysis of still achieving the best effect. Turn up the rate of E/S from 2.5%, 5%, 7.5%, 10%, 12.5% corresponding to the activity is 5.55UI/g, 11.65 UI, 16.1 UI/g, 22.20 UI/g, 27.75UI/g, soluble protein retrieval performance achieved increased from 30.68% to 50.35 percent. Continue to increase the percentage of E/S to 15%, 17.5% of the activity is 33.30UI/g, 38.85UI/g, soluble protein recovery performance does not increase any further which reaches equilibrium. According to the analysis of ANOVA and LSD that values performance recovery of soluble protein in the concentration of 12.5%, 15%, 17.5% have no meaningful difference ($P < 0.05$) at the 95% confidence level. Therefore, we choose the rate of E/S 12.5% for subsequent experiments. This phenomenon is explained as follows: When increasing the amount of enzymes used in the same amount of organic substances, the potential exposure of the enzyme with the substance also increases muscle metabolism reacts to substances that make up the product happens faster at the same time the product form is also more. However, the increased levels of the enzyme use would increase costs for the production process and the product. Because in the same amount of certain substances muscle enzyme levels, increased to a certain level, then all of the peptide bonds in line have been catalytic enzymes assigned to cut off. So, continue to increase the use of enzyme hydrolysis reaction also did not occur and the product form also does not increase further. In addition, the peptide forms back into

enzymatic inhibitory agents [12, 14]. Compare with confronting (not using the enzyme hydrolysis), soluble protein recovery performance won 25.24% while the process of hydrolysis by the enzyme more than ascending from 30.68% to 50.35 percent. Demonstrates the use of enzyme to extraction of protein from soy is suitable. Need surveys to find suitable conditions for enzyme activity to achieve the performance recalls the highest soluble proteins.

3.2.2.3 pH

pH is considered to have a significant influence on factors of enzyme activity. According to the survey results, when the pH was increased from 5, respectively; 5.5; 6; 6.5 the soluble protein retrieval performance achieved increased gradually. And continue to rise from 6.5 to 7 then the recovery performance soluble protein does not increase any further that tends to decrease at pH 7. According to the analysis of ANOVA and LSD, the value of soluble protein recovery performance are statistically different (P 0.05 <) at the 95% confidence level. Based on the graph we notice at pH 6.5 for soluble protein recovery performance gain is the highest (52.48%). So we select the proper pH is 6.5 pH for enzyme activity Flavourzyme hydrolyzed soy protein substances not muscle through heat treatment. This phenomenon is explained as follows: pH can affect the speed of response, due to pH changes affect the ionization state of the functional groups is capable of ionizing take part in Center of enzyme activity, alter the enzyme's ability to link with nature and active changes of enzyme catalysis. Add to that the pH change also changes the physical body ionization or dissociation organic substance. The dissociation of protein molecules in the different pH also changes the nature of the link Centre and muscle enzyme activity leads to catalytic efficiency changes. In addition, nature of the enzymes are proteins should pH also affects the durability of the enzyme.

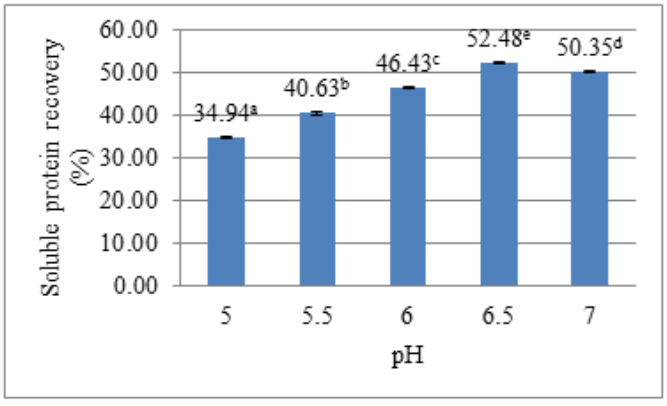

Figure3. Effect of pH to soy protein hydrolysis by Flavourzyme

Note: the values are expressed as the mean of three repetitions ± standard deviation and the same characters (denoted above) then the difference between them is not significant (α = 5%)

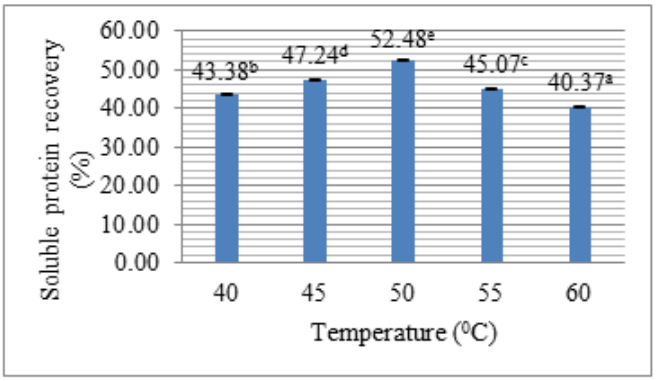

Figure 4. Effect of temperature to soy protein hydrolysis by Flavourzyme

Note: the values are expressed as the mean of three repetitions ± standard deviation and the same characters (denoted above) then the difference between them is not significant (α = 5%)

The temperature has a huge effect on enzyme reaction. Typically rates due to catalytic enzyme will increase when the temperature increases. Indeed, when rising temperatures from 40, 45, 50^{0}C, soluble protein retrieval performance achieved increased gradually. Continued temperature rises from 50^{0}C to 55^{0}C, 60^{0}C, soluble protein recovery performance gain tends to decrease, due to enzyme reaction rate increased only in a certain temperature limit, surpasses that of enzyme reaction speed will decrease and the product formed is also lower. According to the analysis of ANOVA and LSD, then the value of soluble protein recovery performance at different temperatures is significant statistically (P < 0.05) at the 95% confidence level. See soluble protein recovery performance reached the highest in 50^{0}C temperature is 52.48%. This phenomenon is explained as follows: Each enzyme has an optimal temperature range for operation. Different chemical reactions, reaction increases as the temperature increases, while the enzyme only increases in the range of temperature that enzymes have not been denatured real reciprocity. Within the proper temperature, the temperature increases the speed of response, stimulate contacts between enzymes and organic substances, speed up the movement of the structure to increase the mix of enzyme complexes-organic substance, increase the ability to metabolize organic substances constituting products. However, when the temperature rises beyond the optimal activity, enzyme activity will decline. Due to the high temperatures the enzyme is denatured real reciprocity, genetically modified protein structure does not match the original substances, reduces effectively the hydrolysis.

3.2.2.5 Time

In the process of hydrolysis, hydrolysis time is one of the important factors affecting economic performance in production. Hydrolysis time longer then the process of hydrolysis as thoroughly, high hydrolysis. Because the process of hydrolysis requires time to enzymes exposed to the substance and implementation of the peptide links cut in line. Therefore, the longer the time acquisition performance of dissolved proteins reach as high. However, up to a

certain time limit, the effect of hydrolysis process will not change. We see the physical body hydrolysis reaction occurs quickly in the first hour, soluble protein recovery performance increase from 25.24% (model no hydrolysis) to 45.70% (at the time of 60 seconds). Continued hydrolysis time increases from 60 to 120 minutes then dissolved protein retrieval performance achieved increased to 52.57%. Then, despite extended to 150, 180 minutes, soluble protein recovery performance does not increase that reached in equilibrium. According to the analysis of ANOVA and LSD, soluble protein recovery performance at time 0, 60, 90 and 120 the difference is meaningful in 120, 150 and 180 minutes of difference is not statistically significant ($P < 0.05$) at the 95% confidence level. Therefore, we choose the time soy protein hydrolysis by heat treatment by Flavourzyme is 120 minutes. The results are interpreted as follows: the process of hydrolysis occurred rapidly in the early stages, when a large number of peptide bonds in line be cut. Then, the hydrolysis speed decreases when the appropriate peptide bond hydrolysis is much less gradually. After a certain period of time, the peptide bond in line was cut out of the process of hydrolysis of stop and soluble protein content of collected also does not increase any further. In addition, the presence of short peptides also do inhibit the enzyme hydrolysis of effect [12]. These products function as is the nature of competing effectively with the molecules not hydrolysis or partial hydrolysis [14]. Therefore if extended too long hydrolysis not the soluble protein recovery performance gain does not increase further but also affects the quality of the product.

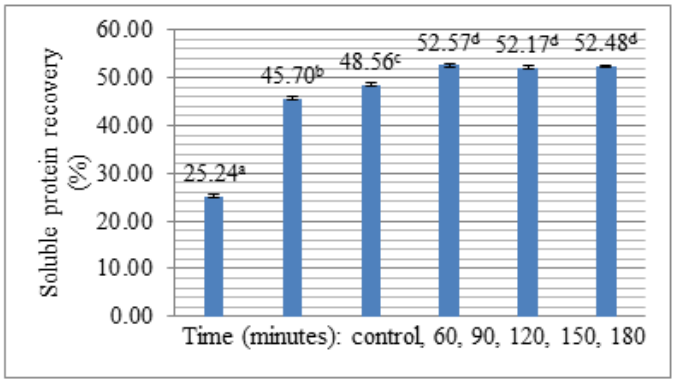

Figure 5. Effect of time to soy protein hydrolysis by Flavourzyme

Note: the values are expressed as the mean of three repetitions ± standard deviation and the same characters (denoted above) then the difference between them is not significant (α = 5%)

3.2.3 Survey results the factors influencing the process of hydrolysis of soybean protein byFlavourzyme in case thermal treatment soy beads

3.2.3.1 Heat treatment

- **Water to heat treatment (water/bean, w/w)**

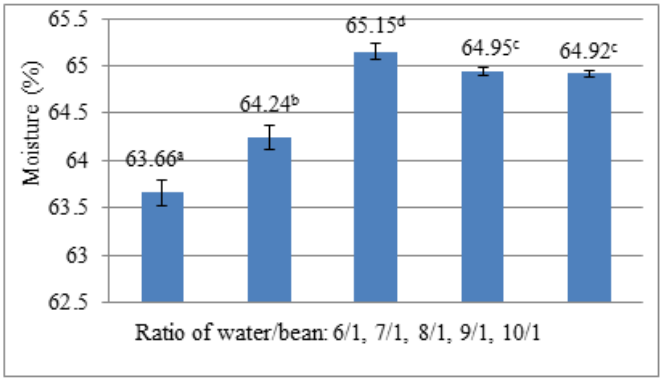

Figure 6. Effect of water used in heat treatment to soybean moisture content

Note: the values are expressed as the mean of three repetitions ± standard deviation and the same characters (denoted above) then the difference between them is not significant (α = 5%)

Turn to change the amount of water used heat treatment of soy beans in pressure cooker under water/bean percentage (w/w) from 6/1; 7/1; 8/1; 9/1; 10/1, then use the Flavourzyme hydrolyzed in conditions: percentage of legumes/water is 1/3; E/S rate is 5%, pH = 7, temperature 50^0C, 180 minutes, the obtained results are as follows: the humidity of the pea ascending correspond to water/bean rate increased from 6/1 to 8/1 and tends to alleviate the continuing rate increases water usage from 8/1 to 10/1. Also soluble protein retrieval performance achieved increased gradually from 42.01 percent to 54.77% corresponding to the rate of water use increased steadily from 6/1 to 8/1 and reach equilibrium if further increased the rate of thermal water from 8/1 to 10/1. This phenomenon is explained as follows: during heat treatment, the pea attracts water, Zhang bloom of humidity changes corresponding to the rate of heat treatment rising steadily. Humidity increases and highest (65.15%) of in water/bean rate is 8/1. Because the process water chemistry hydrate has a certain limit of humidity should be achieved only at a certain value. If it continues to increase the amount of water to Cook, pressure form continues to grow pea can break off and cause a loss of water should the humidity dropped. Pressure cooker pot with lid closed, when the amount of water used heat treatment rising slowly, slightly form also increased but not bailed out be, increases the pressure to pull the temperature in the pot grows. This amount of heat absorbed pea should hurry and become softer. Therefore, the next ground is also easier. At the same time the process of heat treatment also works as a denatured protein, soy protein polypeptide of vessels relaxes the protease enzyme helps easily exposed and the hydrolysis of the peptide matches. In the pressure and temperature to form different then the level of denaturing proteins are also different. Thus, when the amount of water used heat treatment rising gradually then soluble protein retrieval performance achieved is also increasing steadily. However, the level of denaturing also hit at a certain level should have increased the amount of water heat treatment more than how much then can not alter the effect of hydrolysis enzymes means that do not increase the value of soluble

protein recovery performance. Based on the survey results, we select the water/bean rate refers to cooked beans before hydrolysis by Flavourzyme is 8/1.

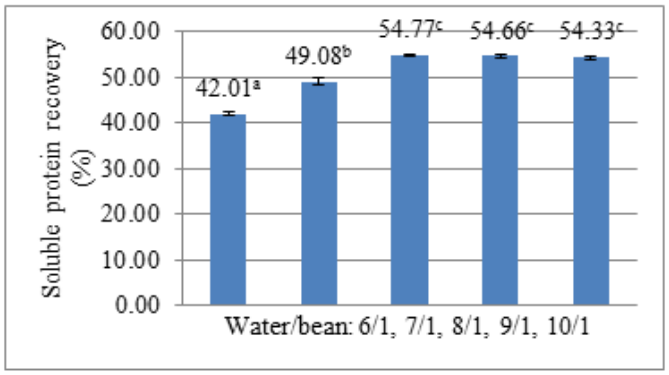

Figure 7. Effect of used water in thermal treatment to soy protein hydrolysis

Note: the values are expressed as the mean of three repetitions ± standard deviation and the same characters (denoted above) then the difference between them is not significant (α = 5%)

- Time of heat treatment

Continue to survey the effects of thermal processing time to the process of hydrolysis in order to find the appropriate time to the process of heat treatment is the most efficient, guaranteeing soft pea and denatured proteins facilitates the process of hydrolysis occurs at its best. Turn heat handle time changes from 10, 15, 20, 25, 30 minutes, the amount of water used heat treatment under water/bean rate is 8/1, the hydrolysis conditions: percentage of legumes/water is 1/3; E/S rate is 5%, pH = 7, 50^{0}C, 180 minutes. The results show: time heat treatment increases the humidity of the soy bean is also ascendant, soluble protein retrieval performance achieved is also ascendant demonstrates the effectiveness of hydrolysis enzymes also increased steadily. Specifically when the increase from 10 to 20 minutes, then pea humidity increase from 61.32% to 65.15% of the soluble protein retrieval performance achieved is 40.64 % to 54.77%. Continue to increase the heat-processing time from 20 minutes to 30 minutes, the humidity tends to reduced soluble protein recovery performance does not change. This phenomenon is explained as follows: heat denature proteins in soy beans but needs some processing time to completely denatured proteins. When the protein reaches the level of the given attribute variable despite extended processing time, the hydrolysis process efficiency is also constant. This means the soluble protein retrieval performance achieved is also unchanged. Add to that time increases heat treatment just as expensive in time and energy, while too long is also not recommended because that will affect the nutritional quality of the product. The phenomenon of increased moisture and tend to decrease is explained similarly as above. So we choose the suitable time to do beans in pressure cooker before hydrolysis is 20 minutes.

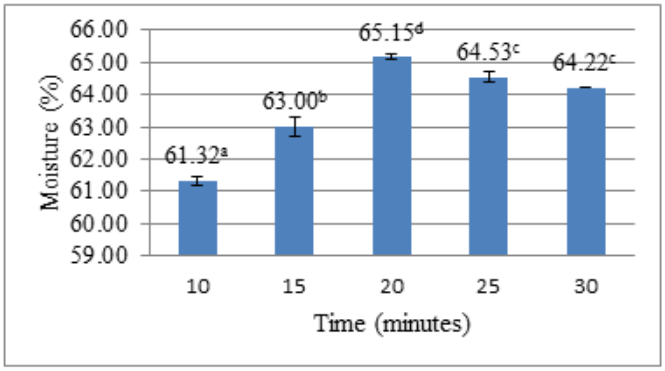

Figure8. Effect of heat treatment time to soybean moisture

Note: the values are expressed as the mean of three repetitions ± standard deviation and the same characters (denoted above) then the difference between them is not significant (α = 5%).

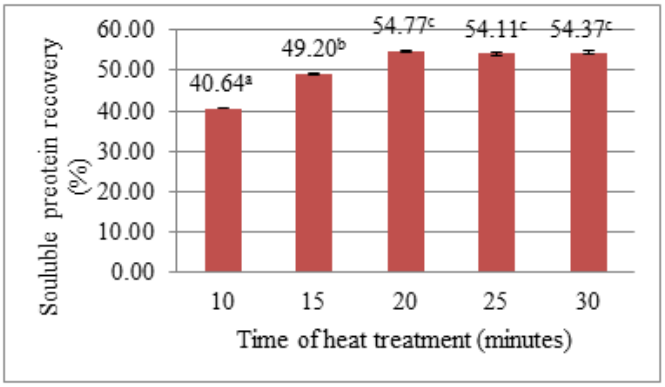

Figure9. Effect of heat treatment time to soy protein hydrolysis by Flavourzyme

Note: the values are expressed as the mean of three repetitions ± standard deviation and the same characters (denoted above) then the difference between them is not significant (α = 5%).

Through the survey above, we notice the influence of thermal modes (water, heat processing time) to the process of soybean seed protein hydrolysis by enzymes quite distinctly. The results obtained are consistent with studies of Morten Fischer et al. (2001) about the correlation between the process of heat treatment and humidity. During heat treatment, where the humidity of the high gain substance over process efficiency hydrolysis occur better. The author undertook 3 hydrolysis of organic substances: soy flour without heat treatment (USBM); soy flour heat treatment achieving high humidity (SBM-H); soy flour heat treatment but reached a low humidity (SBM-L) by four types of enzymes: Alcalase; Flavourzyme; Energex; Biofeed Plus the notice: model SBM-H extraction efficiency achieving 94% higher protein than the 2 remaining samples USBM and SBM-L just reached 89%. The author concludes that the moisture is associated with the vasodilation, denaturing of the protein. When conducting reviews of the extent of the soy protein denatured by DSC method (different scanning

calorimetry), author notice in case of heat treatment quality muscle gain high humidity, the globulin 7S and 11S completely denatured. In contrast, low-humidity heat processing, the level of non-denatured completely. In this study, the author chose mode of heat treatment in autoclave (autoclave) at 125^{0}C, a 20-minute time [10]. In addition, soy beans in protein and carbohydrates linked to form a block of hard to separate, preventing the enzyme activity. Under the effect of heat and the water break the link in the complex proteins and carbohydrates, facilitates access to quality muscle enzyme easier [11]. Review on nutritional aspects that soy is rich in protein supply for human consumption not only of relatively high content of seed proteins that are also fairly balanced amino acid composition. But the nutritional value of soybean seed is limited due to the seeds contain several ingredients nutrition resistance as trypsin inhibitors to prevent the absorption of protein in the human body (Marsman et al, 1997). According to this author, the heat not only denature the protein helps the enzyme hydrolysis of the compound effect that also loads animated nutritional resistance components of improving the nutritional value of soybean seeds. Plus the combination of hydrolysis enzymes contributes to increase the nutritional value of the products [7].

3.2.3.2 Substrate concentration (Bean/water, w/w,%)

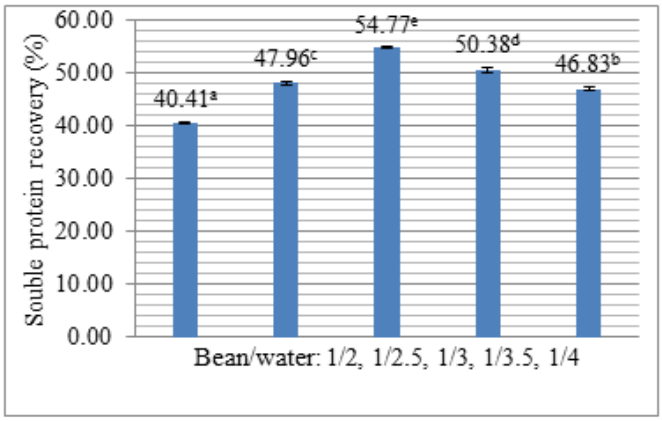

Figure10.Effect of bean/water to to soy protein hydrolysis by Flavourzyme

Note: the values are expressed as the mean of three repetitions ± standard deviation and the same characters (denoted above) then the difference between them is not significant (α = 5%).

Bean rate changes/water turn from 1/4; 1/3.5: 1/3 (correspondingto the dilutionrate descending quality) means that the concentration of the substance increases thesoluble protein retrieval performance achieved increased from 46.83% to54.77%. If it continues to increase the concentration of the substance (bean/waterproportion from 1/3; 1/2.5; 1/2), soluble protein recovery performance gain does notincrease that fell, specifically from 54.77 percent to 40.41 percent. According to theanalysis of ANOVA and LSD then the value recovery performance of different solubleproteins are statistically significant (P < 0.05) at the 95% confidence level. We sawbean/water proportion is 1/3 the soluble protein recovery performance gain isthe highest (54.77 percent).

3.2.3.3 Enzyme/ substrate (E/S, w/w, %)

In turn changes the ratio E/S in sequence from 1%, 3%, 5%, 7% and 9% respectively with the activity: 2.22 UI/g; 6.66 UI/g; 11.10 UI/g; 15.54 UI/g; 19.98 UI/g, the results obtained show that the ratio of E/S increase the soluble protein recovery performance gain also increases. Specific when it increased the rate of E/S from 1% to 7% of the soluble protein recovery performance increase from 35.86 percent to 58.79%. However, when it increased the rate of E/S from 7% to 9% of the soluble protein recovery performance does not increase that reach equilibrium. According to the analysis of ANOVA and LSD soluble protein retrieval performance achieved in the ratio E/S: 7% and 9% is different doesn't make sense statistically. In the ratio E/S 7% going for the soluble protein recovery performance is the highest (corresponding to the activity is 15.54 UI/g) and the ratio of E/S in the next survey. Compare with confronting (not using the enzyme hydrolysis), soluble protein retrieval performance achieved approximately 13.05%. Compared to the model using the enzyme hydrolysis of the soluble protein retrieval performance achieved a lot higher.

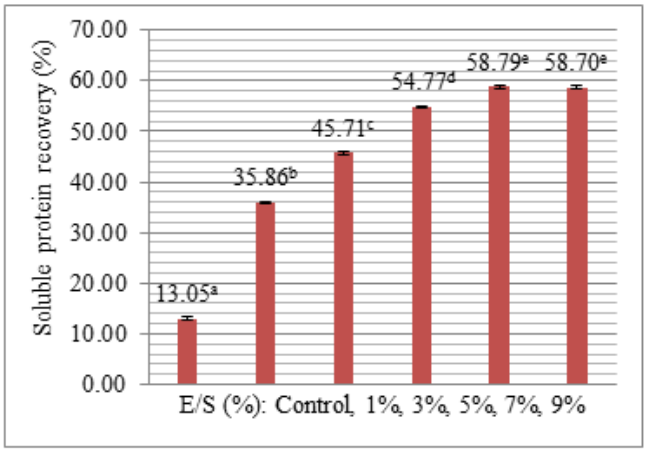

Figure 11. Effect of E/S to soy protein hydrolysis by Flavourzyme

Note: the values are expressed as the mean of three repetitions ± standard deviation and the same characters (denoted above) then the difference between them is not significant (α = 5%).

3.2.3.4 pH

Similar to survey the effects of pH to the process of soybean seed protein hydrolysis in the absence of heat treatment, the pH factor is significant to the enzyme activity. Survey of enzyme activity in the evening activities Flavourzyme like from 5-7 found that: change the pH from 5; 5.5; 6; 6.5 the soluble protein retrieval performance achieved increased, specifically from 45.35 percent to 61.27%. And continue to rise from 6.5 to 7, the soluble protein recovery performance tends to drop and reaches 58.79%. According to the analysis of ANOVA and LSD, then the value of soluble protein recovery performance are statistically different (P 0.05 <) at the 95% confidence level.

Based on the graph we notice at pH 6.5 for soluble protein recovery performance is the highest (61.27 percent). So we select the pH 6.5 is the optimal pH of the enzyme Flavourzyme in subsequent experiments. Each enzyme has a Ph point Max likes to suit every kind of physical mechanism to operate. As according to research by Morten Fischer (2001), pH suitable for Flavourzyme hydrolyzed soy flour over fat is 7 [30], and the pH is suitable for Flavourzyme hydrolyzed SPI is 7.1 (Mo-Nan Zhang, 2011) [9]. In this study, the substance is not soy beans heat treatment and heat-processed, non-fat, the pH is suitable for active Flavourzyme is 6.5. This said, however, that the same is the essence of soy but due to different sample handling process leads to the characteristics of the different substances should pH performance the likes of enzymes also changed. Proven pH is a quite sensitive factors for enzyme activity. Although for any commercial enzyme preparations would offer optimum operating parameters of the manufacturer but must be surveyed again to find suitable hydrolysis conditions for enzyme for the object model are studied.

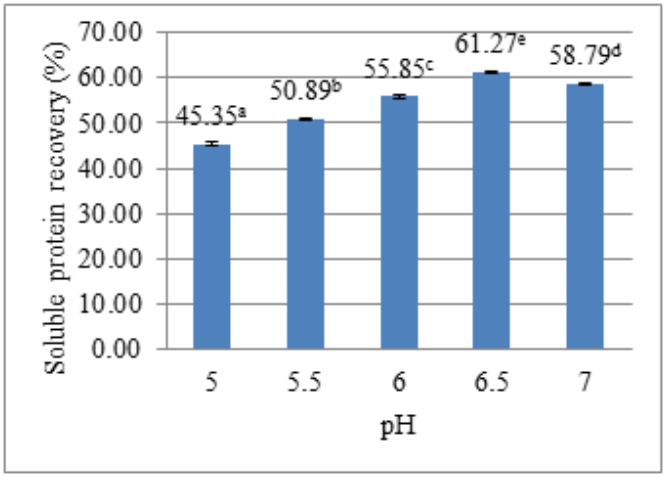

Figure12. Effect of pH to soy protein hydrolysis by Flavourzyme

Note: the values are expressed as the mean of three repetitions ± standard deviation and the same characters (denoted above) then the difference between them is not significant (α = 5%).

3.2.3.5 Temperature

When the temperature rises from 40, 45, 50^{0}C, soluble protein recovery performance increase, in turn from 61.27 percent to 50.10%. And continue to raise the temperature up to 55, 60^{0}C, soluble protein recovery performance gain is reduced. That is because each muscle enzyme hydrolysis activity only good quality within a certain temperature range, high temperature no biochemical reaction velocity increases but also denature any reversible enzyme should affect the efficiency hydrolysis. According to the analysis of ANOVA and LSD, then the value of soluble protein recovery performance at thesetemperatures represent the differences are statistically significant ($P < 0.05$) at the 95% confidence level. 50^{0}C temperature, soluble protein retrieval performance achieved the highest (61.27 percent). Therefore, we chose 50^{0}C as the appropriate temperature for the enzyme Flavourzyme hydrolyzed protein substances muscle activity of soy in this case.

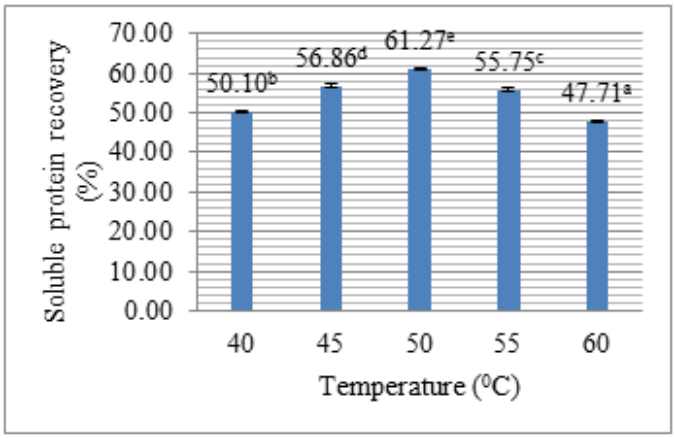

Figure13.Effect of temperature to soy protein hydrolysis by Flavourzyme

Note: the values are expressed as the mean of three repetitions ± standard deviation and the same characters (denoted above) then the difference between them is not significant (α = 5%).

3.2.3.6 Time

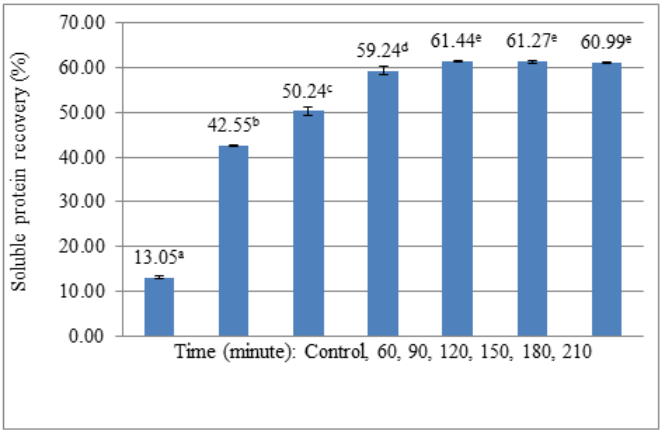

Figure14.Effect of time to to soy protein hydrolysis by Flavourzyme

Note: the values are expressed as the mean of three repetitions ± standard deviation and the same characters (denoted above) then the difference between them is not significant (α = 5%).

Over time the hydrolysis of ascending the soluble protein retrieval performance achieved is also increasing steadily. Basic hydrolysis reaction substances come strong in the early stages, soluble protein recovery performance gain rising from 13.05% (at the time not yet hydrolysis) to 42.55% (in 60 seconds). Demonstrates the enzyme during first contact with appropriate links and easy hydrolysis. Over time the process of hydrolysis is still going on, the links fit tapered, protein obtained still increased but not as powerful as the first time. Continue to rise to hydrolysis time 150 minutes, soluble protein recovery performance gain is

the highest (61.44 percent). Subsequently extended to 180, 210 minutes, soluble protein acquisition performance also does not increase. According to the analysis of ANOVA and LSD protein acquisition performance is dissolved at the time of 150, 180 and 210 minutes of difference is not statistically significant ($P < 0.05$) at the 95% confidence level. Therefore, we choose the time soy protein hydrolysis by Flavourzyme is 150 minutes. Compared with some previous research that found: hydrolysis of protein from soy flour has fat and there through heat treatment by Flavourzyme in conditions: powder/water ratio is 1/10 (w/v), the concentration of enzyme using 1%, pH = 7, 40^0C, 180 min, stirring speed 100rpm, soluble protein recovery performance compared to the original total protein is 65% [26]. In this study, the rate of hydrolysis conditions: beans/water is 1/3; E/S rate is 7%, pH = 6.5, 50^0C, 150 minutes, soluble protein recovery performance reach 61.44%. There is a slight difference is due to different basic physical characteristics (a sample is soy flour made from roasted soy nuts, fat and a sample is non-fat soy beans, cooked) and hydrolysis (enzyme activity, condition of enzyme activity). For example, according to the study by Lee et al. (2001) soluble protein recovery performance reaching approximately 59.5% of the hydrolysis of soybean epidemic has 21% concentration, pretreatment with the heat in combined 95^0C HCl in 1 h and 3 h in the hydrolysis by Alcalase, and continue the hydrolysis by Flavourzyme in 21 h [17]. More study of the Group author Fischer (2001) conducts four types of hydrolysis of soy flour has through various FAT: no heat treatment, heat treatment achieving high humidity, heat treatment achieving low humidity and a product on the market as well through the heat treatment. Soy flour is hydrolyzed (water/powder ratio is 1/9, w/v) at 40^0C, pH = 7, time 16h with the combination of the protease enzyme 2 (Alcalase, Flavourzyme) and 2 carbohydrase (Energex, Biofeed-Plus), the results show that the protein recovery performance are greater than 89% in all samples. However, the highest recovery performance for thermal patterns. In particular, 94% for samples of soy flour processed heat and high humidity reached 92% for commercial product sample [10]. Add to that a combination of enzymes is also effective for protein extraction. However, for the hydrolysis of different enzyme varieties is a complex process, to find the most suitable conditions for all enzymes are performing well recently obtained the desired result.

3.3 Comparision of different soy protein hydrolysis protocols

When examining the factors influencing the process of hydrolysis of protein from soybean by Flavourzyme in two cases no heat treatment and heat treatment are: the absence of heat treatment, the amount of enzyme hydrolysis using more in the case of heat treatment. That's because soy Beans: not yet heat treatment, structure and grain mill be difficult hard to small pea size work as in the case of heat treatment. Add to that the protein is denatured enzyme hydrolysis fluid body should be more difficult to have the amount of enzyme is more than enough to cut off the links accordingly. In summary, the soluble protein recovery performance in case of heat treatment is higher than the absence of heat treatment. Demonstrates the heat helps the process ofhydrolysis soybean protein by Flavourzyme more efficiently, soluble protein recovery performance also increased significantly. Besides saving energy using the enzyme, this means a lot when in terms of economic efficiency. Besides, the heat also do loads of resistant components in soybeans contributed to improve the nutritional value of the hydrolysis products later.

Table 3.Comparision of soluble protein recovery hydrolizedbyFlavourzymein both two cases of non-thernal and thermal method

Method		Non-thermal	Thermal
Soluble protein recovery (%)		52.57 ±0.27	61.44 ± 0.22
Hydrolysis	**Substrate/water (w/w)**	1/6	1/3
	E/S (%)	12.5	7
	pH	6.5	6.5
	Temperature (^{0}C)	50	50
	Time (minutes)	120	120

4 CONCLUSION

Initial research, we find suitable conditions for hydrolysis of soy seeds ingredients with enzymes for protein extraction. Creatingthe basis for the study of soy flour production hydrolysis, the product has high protein content, contains low molecular peptide. This has important implications in terms of nutrition, affecting the ability to absorb the protein in the body and the other biological meaning.

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