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Research Article

Investigation of Enzymatic Optimization by Flavourzyme and Celluclast for Soy Protein Hydrolysate Powder

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ABSTRACT

We optimized hydrolysis conditions of soy protein by Flavourzyme in this case to get the highest yield of soluble protein recovery. The optimal hydrolysis conditions was determined as that pH was 6.6 at temperature 50^{0}C and E/S was 7.8% (17.32UI/g) for 165 minutes, the highest yield of soluble protein recovery was obtained 61.78 ± 0.17%. To support Flavourzyme easily hydrolyzed on substrate, we used Celluclast 1,5L (cellulase) first to disrupt cell walls, release the protein mass inside. However, Celluclast 1,5L did not improve protein extraction. Using E/S (v/w) was 6% (14.28 UI/g) at pH was 5 and 50^{0}C for 120 minutes, and hydrolyzing by Flavourzyme in optimal conditions that got 64.36±0.07%. Compared with independent hydrolysis conditions by Flavourzyme the result increased negligibly. So combination of hydrolyzing by Flavourzyme and heat-treatment was the suitable condition to extract protein from soybeans. The soy protein hydrolysates contained most of peptides with molecular weight among 3.5-8.5kDa at 9.87±0.05% degree of hydrolysis (DH). The tested spray-dried hydrolyzed soybeans product was enough conditions about nutrition, microbiological objective, sensory evaluation and safety for consumers.

Keywords: Hydrolysis, Flavourzyme, Celluclast, Soluble Protein, Optimization.

1. INTRODUCTION

Soy food is quite familiar and used for processing as much as food diet or foods rich in protein such as soy, tofu, chao. Origin from China but now soy has spread out all overtheworld including Vietnam. Currently, scientists have studied, genetic mutations toproduce many new varieties available in manufacturing, food processing and foodprocessing. In Africa, soybeans also helped solve the problem of hunger lack ofnutritious protein. Besides, there are many studies relating to nutritional value andhealth-beneficial effects of soy. So the protein from soy is rated quite high. As in the VCongress of Communist Party of Vietnam has said: "soy needs to bevigorously developed to increase sources of protein for human consumption, for cattle,for the land and became a major export category increasingly important". Therefore, research to find methods of acquisition of soy protein is needed.

Today, the application of enzyme technology in food industry is considered as thecommon technique to extraction, extraction, increased performance recovery ofextracts. Enzymes perform hydrolysis in temperate conditions, the reaction occurs at least create a by-product not only enhance the nutritional value of the product but also provide a lot of value are beneficial to human health. Add to that the food proteinis a source of biologically active peptide but in the form of the root can notcurrently active. In the process of digestion in the stomach, fermented or hydrolyzed byenzymes, they are into peptides having active and affect physiological functions of the body.

Indeed, many studies show that the process of hydrolysis soybean protein byenzyme obtained many peptides in cancer prevention, anti hypertension and reducesblood cholesterol. Therefore, we choose the enzyme protein extraction method is tosoybeans. In order to obtain products with high protein not only enhance thenutritional value of the product but also bring more value to other beneficial for human health.

Flavourzyme is commercial proteases preparations produced from Aspergillusoryzaestrains, produced by fermentation of deep surface without genetic mutations and are used for hydrolysis of protein under the condition of neutral or slightly acid.Flavourzyme is an aminopeptidase, a mixture of peptidase, medium-sized,endopeptidase and exopeptidase.

It is possible to over 70% of hydrolysis of peptidelinkages. Flavourzyme hydrolyzed products created from being mixed with bitterpeptides are diverse, mostly small peptides and amino acids.

The optimal temperaturefor active Flavourzyme (expressing of enzyme blends as well as exopeptidase) in about50-55^{0}C, pH about 5.0-7.0. The optimal pH for enzyme activity instance of theexopeptidase is about 7. The optimum pH was also reduced to bitter 7. Flavourzyme85oC temperature operation is disabled during 5-10 minutes or in 5-second time120^{o}C [5]. Cellulase enzyme that can be produced from molds, bacteria or unicellularorganisms. Cellulase is capable of hydrolysis of cellulose and catalyzed the separatedlinks glucoside in cellulose molecules produced end product is glucose.

The scientific basis of the process of hydrolysis is the process of decomposing achemical compound has a high amount of molecules; with the participation of the country in order to create the new chemical compounds have a lower amount ofmolecules [1].

In the food industry today, to the process of hydrolysis occurs quicklyand the level is high, the producer always use the catalyst. The catalyst is usually the enzymes (biological methods) and non-enzymes (chemical method) [3]. The purpose ofthe technology of hydrolysis process in this research is exploited. Hydrolysis of proteinfrom soybeans into the circuit was shorter peptides have a lower amount of molecules, easy to dissolve in order to obtain products with high content of soluble proteins.

2. MATERIAL AND METHODS

2.1 Raw material

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 andprotease enzyme preparations of Novozymes, Denmark. Conduct the survey as in the content of research topics.

2.2 Research method

The content of research include the:

- Define the initial activity of the enzyme protease (Alcalase and Flavourzyme) and cellulase (Celluclast 1,5L).
- Survey of the process of extraction of protein from soybean by enzymatic method: Celluclast 1,5L with proteases.
- Define the level of hydrolysis and molecular weight of peptides contained in thehydrolysis in the most optimum hydrolysis conditions.
- Trial production and evaluation of the quality of products hydrolyzed soy flour: identifying a number of elements, check only the peroxyde, sensory, microbiological.

2.2.1 Survey select protease preparations suitable for the process of hydrolysis

Preliminary survey conducts two protease preparations (Flavourzyme, Alcalase) to select the proper enzymes with proteolytic process from soybeans. The enzyme usedin thehydrolysis of soy is the heat treatment, in terms of temperature, the optimum pH of the enzyme each (according to the manufacturer's recommendations), along withenzymatic activity in a certain period of time. The objective function is the soluble protein recovery performance while ensuring product sensory properties. From thesurvey, select the enzyme protease in line with the target of research.

2.2.2 Survey of the process of hydrolysis of protein from soybeans in combination celluclast1,5L with protease

After the optimization process is complete hydrolysis of soybean protein by Proteases,we continue to survey the effects of the enzyme Cellulast 1,5L:

Step 1: Use cellulast 1,5L in order to break down the cell wall facilitates proteaseexposure and hydrolysis of organic substance.

Step 2: Keep the hydrolysis of organic substances by proteases.

In this survey, we proceed to examine the effects of the enzyme celluclast 1,5L with two parameters: the rate of enzyme/compound hydrolysis time and muscle, fixedparameters of temperature and pH according to the optimal parameters for which the manufacturer has announced. While conditions of hydrolysis of protease (enzyme/rate of organic matter, pH, temperature, time) according to the

optimal parameters are inthe previous experiments, final assessment is effective hydrolysis when combining twoenzymes by determining soluble protein recovery performance. Final assessment is the degree of hydrolysis and molecular weight of hydrolysis in optimal conditions. Spraydrying and ironing ofsoy hydrolyzed to sensory evaluation of products, check out some nutrients and micro-organisms.

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 to constant weight.-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 improvements.

-Determination of enzyme activity celluclast 1,5L by Miller. -Sensory evaluation method

- Sensory evaluation method of products hydrolyzed soy powder.

2.4 Data analysis

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 errorand software Statgraphics aims to test the reliability of the results obtained fromthese experiments. To determine the results of optimization experiments the influenceof these factors on the objective function, we use the method of the surface meets the RSM (Response Surface Method) and 5.0 Modde software to process the results.

3. RESULTS AND DISCUSSION

3.1Determination of enzyme activity

3.1.1 Enzyme protease

In table 1, the results determined the enzyme activity showed Alcalase enzymes 1630 UI/ml. However, this enzyme which not published the manufacturer's activity should not beevaluated. Enzymes can Flavourzyme is 222 UI/g. Compared to active publication of manufacturer is 500 UI/g, of this enzyme activity lower than announced. This phenomenon is due to the reduced enzyme activity during the storage time.

3.1.2 Enzyme Celluclast 1,5L

In table 2, the results showed that the enzyme Celluclast 1,5L can be 238 UI/ml lower withactivity announced by manufacturers is the 700 UI/g (equivalent to 840 UI/ml). This phenomenon is similar to the enzyme, the enzyme activity Flavourzyme lost duringstorage time.

3.2Optimization of soy protein hydrolysis by Flavourzyme

We optimized the process of hydrolysis of soy protein in the event of heat treatment in order to find the best hydrolysis conditions for enzyme Flavourzyme, aiming to achieve the performance of high soluble protein recovery. Proceed to optimize the four elements under the experimental planning method: the ratio of E/S, pH, temperatureand time (see table 3 & 4). Here are four factors that directly affect enzyme activity. The structure has amind with the objective function is the performance recovery of soluble protein (Y) thatreach the highest. The value is determined as follows: the ratio of E/S (w/w) 7%, pH 6.5;50oC temperature and time is 150 minutes. Optimal testing number in this process is the 31 experiments, including 7 experiments in mind in order to increase the level ofaccuracy of the process. Weights of alternatives is $\alpha = 2\ k/4 = 2$ (where k is thenumber of elements).

Solve planning experiments for the objective function by Modde firmware 5.0 to the method-level Center of rotation, we get the following results:

Through the table 5, we see the variables X1 (ratio E/S), x 2 (pH), X4 (time)that affect the objective function Y (soluble protein recovery performance), due to P<0.05. And these variables in order for work to influence the objective function Y, what about the other variables negatively. Regression equation expressing the relationship ofthe variables in the process of hydrolysis of the following:

$$Y = 61.23+1.57708X_1+1.13208X_2+1.95792X_4–2.02198X_1^2–3.60323X_2^2-2.98823X_3^2–1.89198X_4^2$$

Converted equations in real variables:

$$Y= -198.743 + 2.163503Z_1 + 47.97407Z_2 + 2.98823Z_3 + 0.190297Z_4 – 0.12637Z_1^2-3.60323Z_2^2–0.02988Z_3^2 – 0.00053Z_4^2$$

Where:

- Z_1: is the real variable of parameters ratio E/S (%) of the hydrolysis process.
- Z_2: is the real variable of hydrolysis process pH parameters.
- Z_3: is the real variable of temperature parameters

of the process of hydrolysis (^oC).
$-Z_4$: is the real variable of the time course of hydrolysis (minutes).

Based on Table Analysis of ANOVA (table 6), we noticed: the result is significant statistically ($p < 0.05$) at the 95% confidence level. Add to that the value R2 = 0.965 and Q2 = 0.801satisfies the request of process optimization. Regression equations are represented inthree dimensions and projection to the surface responds will be seen in figure 1-6.
Optimal conditions for the process of hydrolysis were determined by Modde5.0 shows in terms of ratio of E/S (w/w) is 7.8394%; pH 6.6005; temperature 49.6121 ºC; time 165.503 minutes, soluble protein recovery performance is 62.19%. To verifythe accuracy of the value received from the equation of regression we have conducted3 repeated experiments independently based on the optimal value above. Soluble protein retrieval performance achieved 61.78 ± 0.17%. This results in close to thepredicted results from the regression equation.
From the results of optimization process, we select the hydrolysis conditions are as follows:Ratio E/S: 7.8%; pH: 6.6; Temperature: 49.6^oC; Time: 165 minutes

3.3 Survey results the process of hydrolysis soybean protein when combined Celluclast 1,5L with Flavourzyme

Soy bean cell walls are composed of cellulose and hemicellulose, pectin binds withproteins. Pectin duty mounts the plant cell's micell together. The cell is the biggestobstacle when extracted the components hidden inside a cell such as proteins. Figure 7 shows structural clarification of soy beans.
In the soy beans, protein exists mainly in the form of grain in reserve, accounting formost of the volume ofseeds. The major protein blocks easily break the smallprotein. For protein extraction within the county can use mechanical methods (crushing, grinding, etc.) or the enzyme celluclase to break cell walls to release the partof components (including protein) at the same time helps the enzyme protease come into contact with the substance easier [4].
So our basic hydrolysis, soy compound heat-processed in order by celluclast 1,5L first, then continue with Flavourzyme hydrolyzed, to assess the impact of the celluclast 1,5L to the process of hydrolysis of protein from soy. The results obtained specifically as follows:

3..3.1 Ratio of enzyme/substrate (E/S, v/w, %)

Change the Cellulast enzyme rate respectively 1,5L compared to the substance from2%, 6%, 10%, 14% (correspondingto activity is 4.76 UI/ml; 14.28 UI/ml; 23.8 UI/ml;33.32 UI/ml) in the hydrolysis conditions of pH 5, 50^0C, 60 seconds, then continuedthe hydrolysis of organic substances by Flavourzyme in optimized conditions as abovethen we realize: when increasing the rate of E/S from 2% to 6%, then saw the soluble protein recovery efficiency gain has increased in comparison with the case of independent hydrolysis by Flavourzyme, but rises no more. And further increase theratio of E/S to 10%, 14% of the soluble protein recovery performance does not increasethat in equilibrium. According to the analysis of ANONA and LSD are the values at therate of 6%, 10%, 14%, then the difference is not statistically significant ($P < 0.05$) at the95% confidence level. Therefore, we choose the E/S rate is 6% to survey the influence oftime in subsequent experiments (figure 8).

3.3.2 Time

When hydrolysis time increases from 60, 90, 120, 150 and 180 minutes (see figure 9), then found that soluble protein retrieval performance achieved and in 120 minutes is 64.36%.Continue to rise to 150, 180 minutes, soluble protein recovery performance does notincrease further. According to the analysis of ANOVA and LSD are the different valueshave no statistically significant at the 95% confidence level. So, soluble protein retrievalperformance achieved when combined two enzymes Celluclast 1,5L and Flavourzyme is64.36%. However, the performance of this soluble protein recall grows insignificantcompared with the case of using independent hydrolysis by Flavourzyme (61.78percent). Demonstrates, in this case the enzyme Celluclast 1,5L does not have the effect of enzyme hydrolysis Flavourzyme support efficient as original hypothesis.

3.4 Comparison of soy protein extraction protocols

Notice the soy protein hydrolysis by Flavourzyme combined heat treatment; the soluble protein retrieval performance achieved is 61.78% longer when combined add Celluclast 1,5L performance increases to 64.36% (see table 7). Through the survey results showed improved performance is negligible. The purpose of using the enzyme Celluclast 1,5L to break down the cell wall, releasing the nutrients inside, including protein, facilitates the Flavourzyme hydrolyzed contact with substances more easily. However, in the case of using heat to make nine grains of soy, the link between protein and carbohydrate disrupted, as multi-family protein out of this complex enzyme should easily work on the links

in the chain of peptides of soybean protein [9]. Add to that the heat and pressure combined to form works to soften the grain structure that helps the process grind more easily, helping to break the bead structure frees a section containing proteins inside. Thus, using the enzyme Celluclast 1,5L breaking the cell walls support the Flavourzymeproteolytic ineffective. In summary, using more enzymes Celluclast 1,5L not the more time hydrolysis but also adds to the cost of the production process. Not onlydon't add to the effectiveness of the process of hydrolysis, but also affect the quality of the product. The results obtained in this study are similar to Fischer's research (2001): the author's Group concluded that there's no use carbohydrase enzymes aid the process of hydrolysis, protein extraction. Experiments carried out on the substance that is soy flour has been through four fat combined hydrolysis enzyme: proteases (Alcalase, Flavourzyme), carbohydrase (Energex, Biofeed Plus) (AFEB) compared the results with samples only use protease (Alcalase, Flavourzyme) (AF), notice that the AFEB and AF are hydrolysis was 83% protein [8]. Comparison of the results we choose the method of heat treatment to soya beans cooked in combination hydrolysis by Flavourzyme processes suitable for protein extraction from soybean seeds.

3.5Degree of hydrolysis (DH) and peptide molecular mass in hydrolyzing mixture under optimal condition

***3.5.1* Degree of hydrolysis (DH)**

After hydrolysis of organic substances by Flavourzyme in conditions were optimized (E/S rate is 7.8% (corresponding to 17.32 UI/g), pH = 6.60, 49.60 C, 165 minutes), specify the DH of hydrolysis, the results obtained were shown in table 8.

After hydrolysis, hydrolysis fluid obtained with DH's 9.87%. Compared study of Mo-Nan Zhang (2011) hydrolysis of SPI by Flavourzyme in conditions: concentration oforganic substances 2% (w/v, SPI protein concentration initially is 93.8%), the ratio of E/S0.02% (w/v) with activity as 10 UI/g organic matter, pH 7.1, 500C, 120 minutes, the DHof the room after the hydrolysis is 10.02% [7]. Also according to the study by Hrckova et al. (2002) found when using the enzyme Flavourzyme hydrolyzed 1000Lfat soy flour in condition: the concentration of the substance 5%; pH 7, 40^{o}C, 480minutes, DH is very high, obtained approximately 39.5% [6]. DH between differentstudies may be due to differences in basic physical characteristics, enzyme activity,enzyme hydrolysis conditions.

3.5.2 Molecular mass

Currency taking soy protein hydrolysis fluid in optimized conditions above, conduct analysis of electrophoresis, the obtained results are as follows: soy hydrolysed (B) obtaining peptide of molecular weight is concentrated around 3.5-8.5 kDa (see figure 10). Alsotranslated the original raw soybeans yet hydrolysis peptide of molecular then dissolve amajority from 31-38 kDa; 8.5-12 kDa and a few segments have molecular weightpeptide has about 17 kDa, 24 kDa and a few larger sized paragraph 38 kDa, approximately 45-66.2kDa. Through analysis of electrophoresis results found that the process of hydrolysis of soy protein by Flavourzyme produces peptides have a very lowamount of molecules, most are reduced to 3.5-8.5 kDa, which have no translation than hydrolysis.

Compared study of Mo-Nan Zhang (2011) hydrolysis of SPI by Flavourzyme then obtained the majority of peptides having molecular mass from 2-10 kDa in DH = 6.79 percent, while room have no hydrolysis of molecular weight from 10-23 kDa [7]. Also according to Lee (2001), the fat soy flour hydrolysis by AlcalaseFlavourzyme and then obtained the peptides have much lower amounts of molecules, from 360-2000 Da [10]. From the survey results and the results published from the previous research showed that after the process of hydrolysis of soybean protein by enzyme obtained the peptide has a very low amount of molecules. According to numerous studies, the finding that the peptides are active most of the peptides are molecules in very small quantities (Rajapakse et al, 2005) and more efficient in the process of digestion (Webb, 1986) [7]. In particular the following: Peptide has a size of 2-5 kDa, suitable for functional food products, the average size from 1-2 kDa is used for sport objects (Frokjaer, 1994) and the patient (Schmidlet al, 1994), while the smaller sized peptide 1 kDa is used for objects that are allergic (Siemensmaet al., 1993) [2]. In addition, soy protein hydrolysis by protease can generate a number of biologically active peptides are: resistance to cancer (Kennedy, 1995; Lumen, 2005, Jeong et al., 2003); anti-hypertension (Wu and Ding, 2001; Kodera and Nio, 2002; Kitts and Wiler, 2003); lower cholesterol (Bakhitet al, 1994); decrease in plasma triglyceride levels (Iritaniet al, 1996) [2]. In summary, the process of hydrolysis of soy protein by enzymes not only contributes to the extracted protein increases the nutritional value of the product (made up of products containing these peptides, short circuit, low-weight molecules, easy to dissolve), increase absorption in the digestion of soy protein in the body and also many other meanings (such as finding peptides have active) need be studied in more detail.

3.6Quality of protein hydrolysate powder

Through the survey, we realize results to factors affecting the process of hydrolysis of soy protein in order to find suitable conditions for extraction of protein from soy beans. Since then, currency translation of hydrolysis, producing spray driers tested hydrolysis soybeanflour. Then flour analysis identified a number of components, test some indicators aboutmicrobiology, peroxyde, the results obtained are shown in table 9& 10.

Products with low humidity (5.27 percent) so we can extend the storage time withoutcompromising product quality. The product has a high protein content (47.19%) ascompared to a dry substance content, this means a lot when in terms of nutrition.Products with high fat content makes up 22.38% compared to a dry substancecontent, however the high no peroxyde (0.26 meq/kg) and is within the thresholdallows. Compared to the ISO 7597: 2007 oxidation of fats in the grease, thelimit is 10 meq/kg. However, it should also be noted when preserved. Products meetrequirements for micro-organisms under the current regulatory standards.

We conduct Expert Council consists of 5 people to sensory evaluation of soy flour whendissolved in water, according to four criteria: color, smell, taste and status according tothe method for the point. The results obtained were as follows: analysis of ANOVA to evaluate the differences between the expert and the difference between the 3repetitions according to the norms (the color, smell, taste, status) of each sample shallhave a p-value > 0.05, proven results garner the difference is not statisticallysignificant. Sensory evaluation demonstrated the norms of the experts are the same andbetween sensory evaluations is no different. When comparing the sample (the ratio ofmixing powdered sugar and water) are found between the different patterns of (p-value < 0.05), and the target color, the smell, the same status (p-value > 0.05). Alsoreviews the difference between professionals and between repetitions is the same.Sensory evaluation results obtained samples of 1 g of powder mixing rate: 1, 5 g sugar:10 ml of water reaching the highest score (see table 11).

In general, when conducting sensory evaluation, indicator color has a point about 4-5, while the targets of smell, taste, the state has ranged from about 3-4, total scoremultiplied by the weight is 3.8 > 4.07, a total score of 16.1, meet the requirements set out. Compared to a scale rating of quality of ISO 3215-79, then this product reaches the kind of pretty. Hydrolysis of soy flour products meets all the requirements forsensory, microbiological, nutrition, safety for the user.

4. CONCLUSION

The hydrolysis process survey in combination Celluclast 1,5L with Flavourzyme thenfound Celluclast 1,5L does not have the effect of enzyme protein extractionFlavourzyme support. Soluble protein retrieval performance achieved 64.36 ± 0.07%rise is negligible compared to the case of independent hydrolysis by Flavourzyme(61.78 ± 0.17 percent). Translate hydrolysis soybean obtained according to theprocedure that DH is 0.05% ± 9.87 and contains most of the peptide molecular mass from 3.5-8.5 kDa. Finally, we conducted product trial manufacturing of soy hydrolyzedby spray drying room hydrolysis. Powder products obtained have relatively highprotein content (accounting for 44.7%), the microbiology and indicators satisfactoryperoxyde, safe for users. In this study, after the hydrolysis of peptides obtained havevery low energy molecules, proved capable of obtaining valuable peptide biology. So can research isolate the peptides having biodiversity, studythe active of this peptideand application possibilities as food and medicine. Hydrolysis starch products obtainfairly high fatty content in soybeans mostly unsaturated fatty acids should need to findmethods of extending storage time oxidation product without being fat.

Table 1.Enzyme activity of Flavourzyme andAlcalase

Enzyme	$OD_{sample}(A^0)$	$OD_{control}(A^0)$	Tyrosine concentration (µmol/ml)	Dilution ratio	Time (minutes)	Activity (UI/g-ml)
Flavourzyme	0.1716	0.0216	0.1392	2000	10	222 ± 0.45
Alcalase	0.1233	0.0134	0.1019	20000	10	1630 ± 1.48

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

Table 2
Enzyme activity of Celluclast 1,5L

OD_{sample}	$OD_{control}$	Glucose (mg/ml)	Dilution ratio	Time (minutes)	Activity (UI/ml)
0.088	0.039	0.12866	2000	30	238 ± 3.84

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

Table 3
Experimental parameter of the optimization

Parameter	-α	-1	0	1	+α
X_1(E/S) (%)	3	5	7	9	11
X_2(pH)	5.5	6	6.5	7	7.5
X_3 (^{o}C)	40	45	50	55	60
X_4 (minutes)	90	120	150	180	210

Table 4
The matrix of planning the structure of secondary care, the four elements

X_1	X_2	X_3	X_4	E/S (w/w)	pH	Temperature (^{0}C)	Time (minutes)	Soluble protein recovery (%), Y
-1	-1	-1	-1	5	6	45	120	46.22
1	-1	-1	-1	9	6	45	120	48.33
-1	1	-1	-1	5	7	45	120	48.25
1	1	-1	-1	9	7	45	120	53.15
-1	-1	1	-1	5	6	55	120	45.69
1	-1	1	-1	9	6	55	120	47.71
-1	1	1	-1	5	7	55	120	48.25
1	1	1	-1	9	7	55	120	49.73
-1	-1	-1	1	5	6	45	180	50.48
1	-1	-1	1	9	6	45	180	51.11
-1	1	-1	1	5	7	45	180	51.67
1	1	-1	1	9	7	45	180	55.96
-1	-1	1	1	5	6	55	180	49.34
1	-1	1	1	9	6	55	180	50.47
-1	1	1	1	5	7	55	180	51.72
1	1	1	1	9	7	55	180	55.37
-2	0	0	0	3	6.5	50	150	49.75
2	0	0	0	11	6.5	50	150	58.57
0	-2	0	0	7	5.5	50	150	47.23
0	2	0	0	7	7.5	50	150	48.44
0	0	0	-2	7	6.5	40	150	51.26
0	0	0	2	7	6.5	60	150	49.33
0	0	0	-2	7	6.5	50	90	50.13
0	0	0	2	7	6.5	50	210	59.23
0	0	0	0	7	6.5	50	150	61.13
0	0	0	0	7	6.5	50	150	61.09
0	0	0	0	7	6.5	50	150	61.23
0	0	0	0	7	6.5	50	150	61.41
0	0	0	0	7	6.5	50	150	61.35
0	0	0	0	7	6.5	50	150	61.36
0	0	0	0	7	6.5	50	150	61.04

Table 5
Effects of independent variables to the soluble protein recovery performance

Factor	Value of regression equation	Standard deviation	P	Conf. int(±)
Constant	61.23	0.524128	6.94684e-025	1.1111
X_1	1.57708	0.283062	4.2143e-005	0.600063
X_2	1.13208	0,.283062	0.00103329	0.600064
X_3	-0.447921	0.283062	*0.13312*	0.600064
X_4	1.95792	0.283062	3.46726e-006	0.600063
X_1^2	-2.02198	0.25932	7.72475e-007	0.549733
X_2^2	-3.60323	0.25932	2.39362e-010	0.549733
X_3^2	-2.98823	0.25932	3.69295e-009	0.549733
X_4^2	-1.89198	0.25932	1.79367e-006	0.549733
X_1X_2	0.526876	0.346678	*0.148079*	0.734925
X_1X_3	-0.228124	0.346678	*0.519877*	0.734925
X_1X_4	-0.0506227	0.346678	*0.885729*	0.734925
X_2X_3	-0.0643733	0.346678	*0.855026*	0.734925
X_2X_4	0.118126	0.346678	*0.737734*	0.734925
X_3X_4	0.140629	0.346678	0.690375	0.734924

Table 6
Analysis of variance Anova of optimization experiments

Y	DF	SS	MS	F	P	SD
Total	31	88283	2847.84			
Constant	1	87397.3	87397.3			
Total Corrected	30	885.711	29.5237			5.43357
Regression	14	854.943	61.0674	31.7567	0.000	7.81456
Residual	16	30.7676	1.92297			1.38671
Lack of Fit	10	30.6382	3.06382	142.064	0.000	1.75038
Value of standard error	6	0.129399	0.0215665			0.146855
N = 31	$Q^2 = 0.801$					
DF = 16	$R^2 = 0.965$					

Where R^2: coefficient of determination; SS: sum of squares; DF: degrees of freedom;MS: mean square; F: F-value; p: p-value. The value F, p reliability at the 95%.

Table 7
Comparison of soluble protein recovery by Flavourzymeand combination of Celluclast1,5L +Flavourzyme

Treatment method	Soluble protein recovery (%)
Heating+ Flavourzyme	61.78 ± 0.17
Heating + Celluclast 1,5L + Flavourzyme	64.36 ± 0.07

Table 8
DH of hydrolyzing mixture by Flavourzyme

Volume of NaOH 0.1N (ml)	DH (%)	Average DH (%)
3	9.88	9.87±0.05
2.98	9.82	
3.01	9.92	

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

Table 9
Compostitions in protein hydrolysate powder

Composition	Value	Based on dry matter (%)
Lipid (%)	21.2	22.38
Carbohydrate (%)	16.4	17.31
Moisture (%)	5.27	-
Total sugar (%)	0.59	0.62
Protein (%)	44.7	47.19
Ash (%)	12.4	13.09
Peroxyde (meq/kg sample)	0.26	-

Table 10
Microorganism in protein hydrolysate powder

Microorganism	Value	Limit	Note
Coliforms (cfu/g)	<10	<3	QC VN 8-3: 2012/BYT
Salmonella (/25g)	Not detected	0	

Table 11
Sensory score of protein hydrolysate powder

Criteria	Description	Score	Emphazised score	Total score
Color	Yellow powder characteristic of soybean The dough when mixing into the water also has a characteristic yellow soybeans	4.7	1.41	16.1
Flavor	Powder and mixed with water line when the translation has a characteristic smell of soy	3.7	0.925	
Taste	The powder is dissolved in water, add sugar mixed with medium sweetness but lessfattening	3.9	0.975	
Appearance	The powder dissolves into the water, mixing more sugar: liquid melt, there is very littleresidue	3.8	0.76	

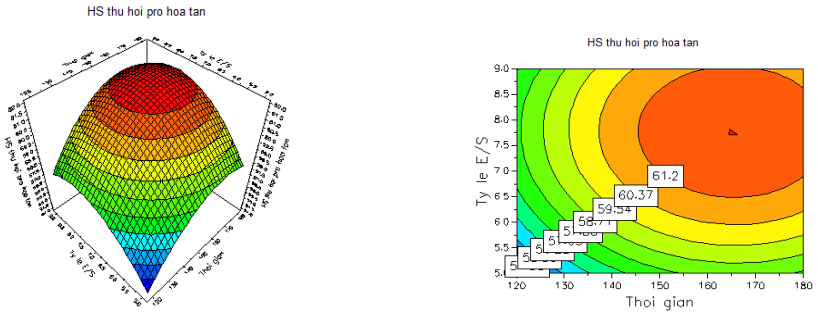

Figure 1
Effect of E/S and time to soluble protein recovery

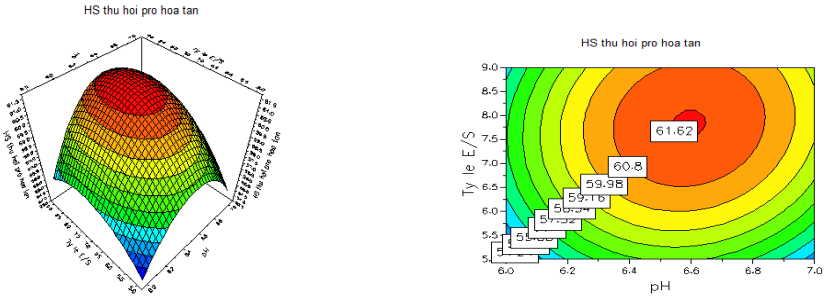

Figure 2
Effect of E/S and pH to soluble protein recovery

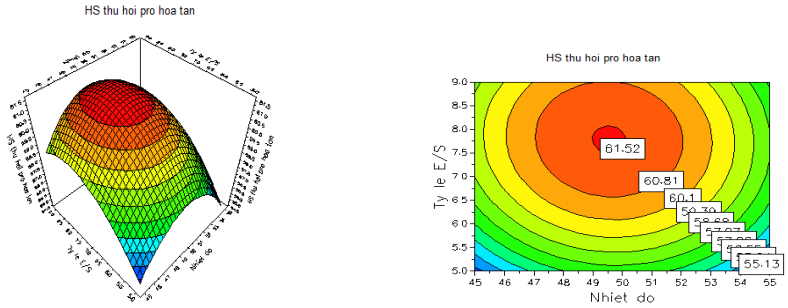

Figure 3
Effect of E/S and temperature to soluble protein recovery

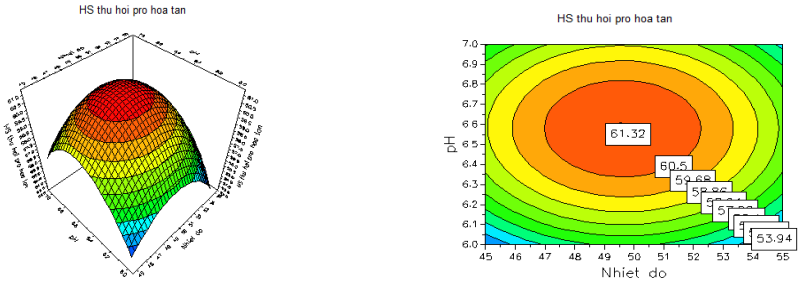

Figure 4
Effect of temperature and pH to soluble protein recovery

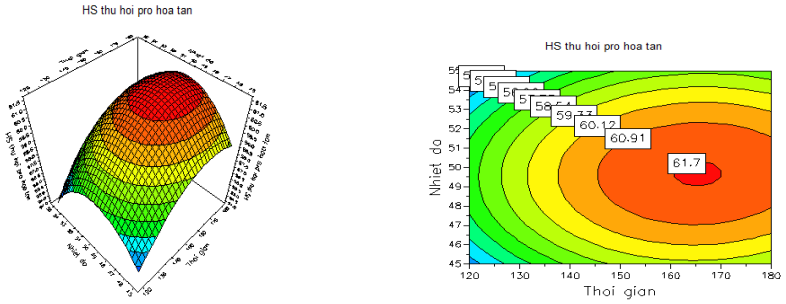

Figure 5
Effect of temperature and time to soluble protein recovery

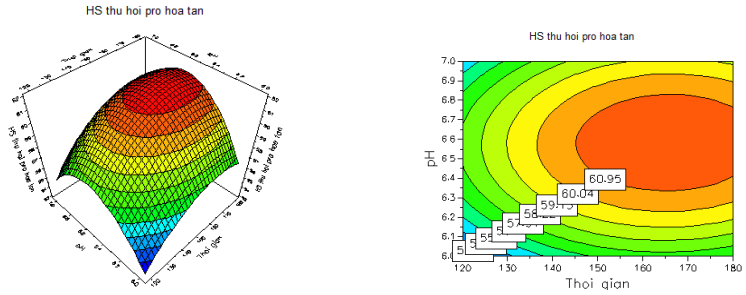

Figure 6
Effect of pH and time to soluble protein recovery

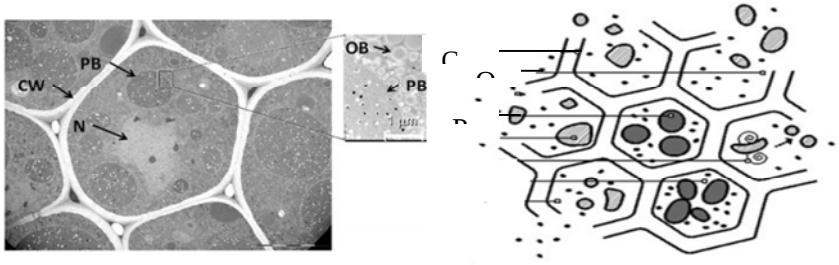

Figure 7
Structure of soybean cell wall [5]
CW: cell wall; PB: protein body; OB: Oil body; N: nucleus

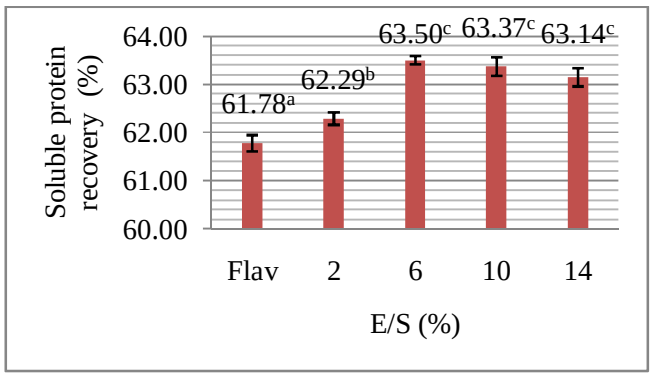

Figure 8
Effect of E/S to soy protein hydrolysis
Note: The values are expressed as the mean of three repetitions ± standard deviationand the same characters (denoted above) then the difference between them is not significant (α = 5%)

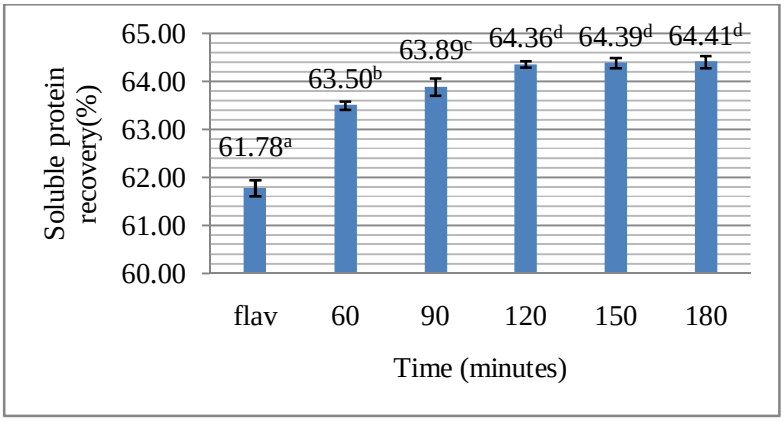

Figure 9
Effect of time to soy protein hydrolysis
Note: the values are expressed as the mean of three repetitions ± standard deviationand the same characters (denoted above) then the difference between them is notsignificant (α = 5%)

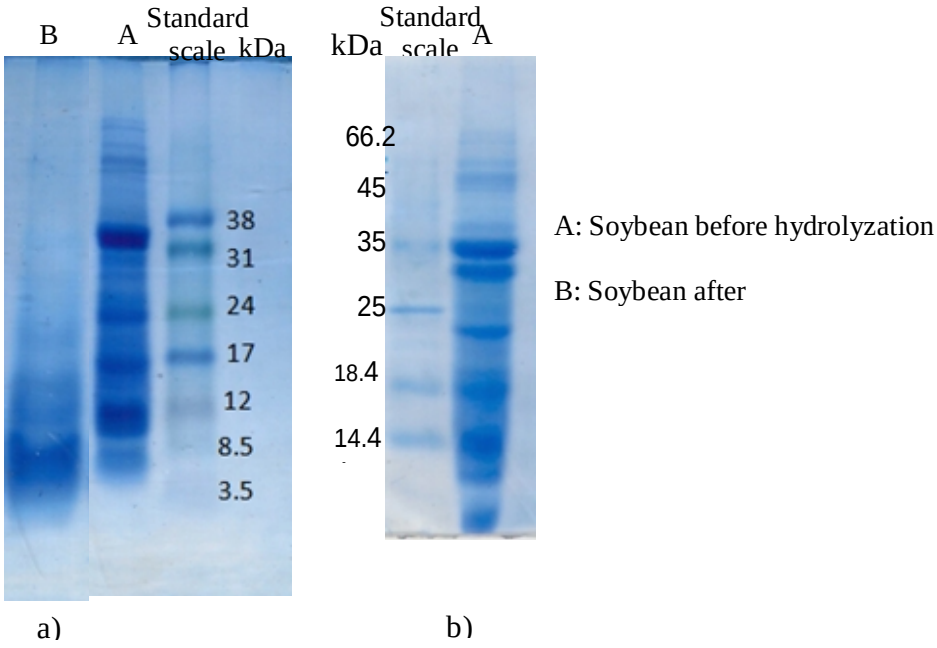

Figure 10
The result analysis of electrophoresis Flavourzyme hydrolyzed soy bean mixture

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