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Research Article

Optimization of Enzymatic Hydrolysis for Soy Milk under Experimental Planning

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ABSTRACT

The process optimization is carried out for soy milk under experimental planning and the results obtained are as follows: enzyme activity is 30.05 UI/g raw beans, pH 6.6,a temperature of 49.8°C, time is 133 minutes. Then proceed to compare methods of hydrolysis by enzymes Flavourzyme with water extraction method of performance recovered soluble protein, molecular mass. The results showed Flavourzyme hydrolyzed by enzyme method for soluble protein recovery performance reached 52.589% higher 9.352% compared to water extraction method, low molecular weight about 10-31kDa while water extraction method of molecular weight is 15-150kDa.

Keywords: Optimization, Soy milk, Enzyme activity, water extraction, Experimental planning.

1. INTRODUCTION

Soy milk (soymilk or soya milk) is a plant milk produced by soaking dry soybeans and grinding them with water. A traditional staple of Asian cuisine, soy milk is a stable emulsion of oil, water and protein, and contains about the same proportion ofprotein as cow's milk. The coagulated protein can be made into tofu, just as cow's milk can be made into cheese. Soy milk can be produced at home using a soy milk machine

Soy milk is a complete protein and has about the same amount of protein as cow's milk; it can replace animal protein and other sources ofdietary fiber, vitamins and minerals[12]. Soy milk contains little digestible calcium because calcium is bound to the bean's pulp, which is indigestible by humans. To counter this, manufacturers enrich their products with calcium carbonate. Unlike cow's milk, soy milk has littlesaturated fat and no cholesterol.

Soy products contain sucrose as the basic disaccharide, which breaks down into glucose and fructose. Since soy does not containgalactose, a product of lactose breakdown, soy-based infant formulas can safely replace breast milk in children with galactosemia. Like lactose-free cow's milk, soymilk contains no lactose, which makes it an alternative for lactose-intolerant people.

It has been suggested that soy consumption is associated with a reduction in low-density lipoprotein ("bad cholesterol") and triglycerides[3]. Research has refuted claims that soy affects bone mineral density[4]. Research has found no link between soy and increased estrogen levels in men, although studies thus far have been limited in duration[9]. Soy protein is increasingly used in extended meat products and dairy type products due to the presence of high quality proteins with excellent functional properties. However, it has been shown to inhibit iron bioavailability because of phytic acid present in the protein[11].

There are several noticeable studies mentioned to soy milk hydrolysis.

AllaouaAchouri et al. (1998) examined the enzymatic hydrolysis of soy protein isolate and effect of succinylation on the functional properties of resulting protein hydrolysates. Commercial defatted soy meal was solubilized in an aqueous solution at pH 8.5 to prepare a soy protein isolate (SPI) with 90.45% protein and 95% solubility. Soy protein hydrolysate (SPH) was obtained by enzymatic hydrolysis of the SPI using a neutral proteinase at different degrees of hydrolysis (DH=4, 6, 8 and 10). A previous heat treatment of native SPI

at 80°C for 10 and 30 min caused a gradual dissociation and/or unfolding of some fractions of the soy protein leading to a decrease in high molecular weight fractions. Gel filtration chromatography of SPH with DH=8 indicated that the soluble fraction consisted mostly of low molecular weight peptides having a molecular weight less than 12.5 kDa. Combined hydrolysis and succinylation greatly increased protein solubility and caused marked changes in other functional properties depending on the degree of modification[1].

A.M. Calderón De La Barca et al. (2000) investigated the enzymatic hydrolysis and synthesis of soy protein to improve its amino acid composition and functional properties. Soy protein was enzymatically modified and ultrafiltred, and functional properties were evaluated. After enzymatic hydrolysis, hydrolysate (20 g/100 mL) was incubated with chymotrypsin and glycerol at 37 ^{o}C. Different methionine methyl-ester concentrations, pH, and time were tested. Amino acid composition and functional properties of ultrafiltrated fractions (FI>10, 10>FII>3, and 3>FII>1 kDa) were evaluated. Optimum hydrolysis conditions were 12 h and 50 ^{o}C, and those of synthesis were 0.07585 g Met/g, pH 7, and 3 h, binding 2.2% to5% methionine. Fractions under 10 kDa presented 100% solubility and the best clarity. High-methionine fractions had higher foam volume, lower emulsifying capacity and hydrophobicity. Modified hydrolysates have a potential for use in soluble high nutritional products[2].

Martina Hrckova et al. (2002) conducted the enzymatic hydrolysis of defatted soy flour by three different proteases and their effect on the functional properties of resulting protein hydrolysates. Commercial defatted soy flour (DSF) was dispersed in distilled water at pH 7 to prepare 5% aqueous dispersion. Soy protein hydrolysates (SPH) were obtained by enzymatic hydrolysis of the DSF using three different proteases (Flavourzyme 1000 L, No-vozym FM 2.0 L and Alcalase 2.4 L FG). The highest degree of hydrolysis (DH 39.5) was observed in the presence of protease Flavourzyme. SPH were used for measuring functional properties (foaming stability, gelation). Treatment with Flavourzyme improved foaming of proteins of DSF. Foaming stability was low in the presence of Novozym. Proteases treated DSF showed good gelation properties, mainly in the case of treatment with Flavourzyme. SDS-PAGE analysis showed that after enzyme addition to the 5% aqueous dispersion of DSF each enzyme degraded both β-conglycinin and glycinin. In general, the basic polypeptide from glycinin showed the highest resistance to proteolytic activity. The most abundant free amino acids in the hydrolysates were histidine (30%), leucine (24%) and tyrosine (19%) in the case of the treatment with proteases Alcalase and Novozym, and arginine (22.1%), leucine (10.6%) and phenylalanine (12.9%) in the case of the treatment with Flavourzyme[7].

Miroljub B. Barac et al. (2006) demonstrated the effect of limited hydrolysis on traditional soy protein concentrate. The influence of limited proteolysis of soy protein concentrate on protein extractability, the composition of the extractable proteins, their emulsifying properties and some nutritional properties were investigated. Traditional concentrate (alcohol leached concentrate) was hydrolyzed using trypsin and pepsin as hydrolytic agents. Significant differences in extractable protein composition between traditional concentrate and their hydrolysates were observed by polyacrylamide gel electrophoresis (PAGE) and by SDS-PAGE. All hydrolysates showed better extractability than the original protein concentrate, whereas significantly better emulsifying properties were noticed at modified concentrates obtained by trypsin induced hydrolysis. These improved properties are the result of two simultaneous processes, dissociation and degradation of insoluble alcohol-induced protein aggregates. Enzyme induced hydrolysis had no influence on trypsin-inibitor activity, and significantly reduced phytic acid content[8].

Lin Chen et al. (2011) showed the effects of ultrasound pretreatment on the enzymatic hydrolysis of soy protein isolates and on the emulsifying properties of hydrolysates. Soy protein isolate (SPI) was modified by ultrasound pretreatment (200 W, 400 W, 600 W) and controlled papain hydrolysis, and the emulsifying properties of SPIH (SPI hydrolysates) and USPIH (ultrasound pretreated SPIH) were investigated. Analysis of mean droplet sizes and creaming indices of emulsions formed by SPIH and USPIH showed that some USPIH had markedly improved emulsifying capability and emulsion stabilization against creaming during quiescent storage. Compared with control SPI and SPIH-0.58% degree of hydrolysis (DH), USPIH-400W-1.25% (USPIH pretreated under 400W sonication and hydrolyzed to 1.25% DH) was capable of forming a stable fine emulsion (d_{43} = 1.79 μm) at a lower concentration (3.0% w/v). A variety of physicochemical and interfacial properties of USPIH-400W products have been investigated in relation to DH and emulsifying properties. SDS−PAGE showed that ultrasound pretreatment could significantly improve the accessibility of some subunits (α-7S and A-11S) in soy proteins to papain hydrolysis, resulting in changes in DH, protein

solubility (PS), surface hydrophobicity (H_0), and secondary structure for USPIH-400W. Compared with control SPI and SPIH-0.58%, USPIH-400W-1.25% had a higher protein adsorption fraction (F_{ads}) and a lower saturation surface load (Γ_{sat}), which is mainly due to its higher PS and random coil content, and may explain its markedly improved emulsifying capability. This study demonstrated that combined ultrasound pretreatment and controlled enzymatic hydrolysis could be an effective method for the functionality modification of globular proteins[6].

Xiang Dong Sun (2011) verified the enzymatic hydrolysis of soy proteins and the hydrolysates utilisation. Soy protein hydrolysates were primarily used as functional food ingredients, flavour and nutritious enhancers, protein substitute, and clinical products. Conditions for hydrolysis were usually mild, whereas recently high pressure treatment attracted more interest. Degree of hydrolysis (DH) was usually between 1% and 39.5%. The main problem associated with proteolytic hydrolysis of soy protein was production of bitter taste, hydrolysates coagulation and high cost of enzymes. Bitterness reduction can be achieved by control of DH, selective separation of bitter peptides from hydrolysates, treatment of hydrolysates with exo-peptidases, addition of various components [adenosine monophosphate (AMP), some amino acids, monosodium glutamate (MSG), etc.] to block or mask the bitter taste, and modification of taste signalling. Hydrolysates coagulation can be resolved by selecting appropriate enzymes and by applying immobilisation technology the production cost can be reduced. Enzymatic hydrolysis also enhances bioactivity of soy proteins through conversion of glycosides to aglycones, increasing antioxidant and immunoregulatory properties[13].

Fuh-Juin Kao et al. (2011) proved the optimization of enzymatic hydrolysis conditions for producing soy protein hydrolysate with maximum lipolysis-stimulating activity. The optimum hydrolysis conditions of 2.5% (w/v) soy protein isolate (SPI) with 1% (w/w of SPI) Flavourzyme® for increasing glycerol release in mature 3T3-L1 adipocytes were investigated by response surface methodology (RSM). Higher glycerol release indicated higher lipolysis-stimulating activity. The independent variables were hydrolysis time (HT) 19.2 - 220.8 min, pH 5.32 - 8.68 and reaction temperature (RT) 33.2 - 66.8°C. Based on the response surface and contour plots, the optimum hydrolysis of SPI with Flavourzyme® for maximizing the glycerol release in the cells occurred at pH = 7.12, RT = 48.77°C and HT = 124.85 min. The F-value for lack of fit was not significant ($p > 0.05$), so the second order model was appropriate for describing the response surface. In addition, the model had a satisfactory coefficient of R2 (= 0.935) and was verified experimentally[5].

Maomao Zeng et al. (2013) studied the improving the foaming properties of soy protein isolate through partial enzymatic hydrolysis. The effect of partial enzymatic hydrolysis of soy protein isolate (SPI) on its foaming properties is investigated in this study. Enzymes of different origin, including papain, alcalase, and pancreatin, were used. Foaming properties (foaming capacity and foam stability) were measured and their relationships with physicochemical characteristics such as degree of hydrolysis, molecular weight of hydrolysates, and surface tension were investigated. Papain hydrolyzed SPI hydrolysates were found to be the best in terms of improved foaming capacity and foam stability. Molecular weights of SPI hydrolysates obtained by papain and alcalase hydrolysis were mainly in the range of 5 kDa to 30 kDa, while those hydrolyzed by pancreatin had molecular weight above 50 kDa. Foaming capacity was found to correlate well with the relative abundance of hydrolysate in the molecular weight range of 5 kDa to 10 kDa ($r = 0.84$, $p < 0.05$). Surface hydrophobicity was found to correlate negatively ($r = -0.89$, $p < 0.05$) with foaming capacity of SPI hydrolysates. Surface tension values of SPI hydrolysates produced by all enzymes were significantly lower than ($p < 0.05$) compared to that of SPI. The surface tension of pancreatinhydrolyzed SPI hydrolysates was lower than the surface tension of those hydrolyzed by papain and alcalase. The surface tension of pancreatinhydrolyzed SPI hydrolysates decreased more rapidly with time compared to the rest. These findings will provide better understanding of how best to carry out the partial hydrolysis of SPI using various enzymes in order to improve its foaming properties[10].

Mo-Nan Zhang et al. (2014) investigated theiron binding capacity of dephytinised soy protein isolate hydrolysate as influenced by the degree of hydrolysis and enzyme type.This present study investigated the effects of dephytinise from soy protein isolate (SPI) on iron binding capacity and degree of hydrolysis. Also the effects of enzyme type and degree of hydrolysis on iron binding capacity were studied. It was demonstrated that phytase and anion exchange resin could remove effectively the phytate from SPI. The dephytinise would decrease the degree of hydrolysis of SPI. The enzyme type and degree of hydrolysis influenced significantly the iron binding capacity of the hydrolysate. Flavourzyme might be the best choice for producing peptides with

iron binding capacity from SPI and middle degree of hydrolysis would be benefitable to this process[11].
Main scope of our research is to use the method of optimization by experimental planning to choose the active enzymes, pH, temperature, 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. 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, PhuNhuan District).

2.2 Method

2.2.1 Optimize enzyme activity, pH, temperature and time of hydrolysis by experimental planning method

Purpose: examine the influence and activity of enzymes Flavourzyme, pH, temperature and time of hydrolysis to soluble protein recovery performance. From there determine the enzyme activity, pH, temperature and time optimal hydrolysis to soluble protein recovery performance of hydrolysis is the highest.
Methods: experiments were done by the method of orthogonal experiment planning the four elements, the planning according to the CCC model. The software used isModde 5.0. The objective function is the soluble protein recovery performance of hydrolysis. The optimum value is the point at which the soluble protein recovery performance of hydrolysis according to the equation of regression reaches maximum value.

2.2.2 Survey of soy protein extraction processes with water

Purpose: examine the effects of bean: domestic protein extraction process by water. Prepare materials: soybeans are soaked in hot water at 40^{o}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. Soybeans will be grinding with water at ambient temperature. Proceed to filter the currency pulled translates soy. Then translate the soybeans will be determining soluble protein recovery performance. From there pick out bean: water ratio for soluble protein recovery performance. 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.

2.2.3 Comparison and reviews

From the results of optimization method of hydrolysis soybean protein by enzymes and protein extraction methods Flavourzyme soybeans in water, conduct comparison and evaluation of two methods on the recovery of performance soluble protein and molecular weight.

2.3 Analytical methods

-Moisture content: by drying sample to constant weight.
-Concentration of total protein: by Kjeldahl method (AOAC 984.13, 2000).
- Concentration of total lipid: by Soxhlet method (AOAC 960.39, 2000).
- Enzyme activity: by Anson method.
- Concentration of soluble protein: by Lowry method.
- The degree of hydrolysis of DH: by by maintaining the hydrolysis patterns at a constant pH value after a certain hydrolysis with NaOH 0, 1N.
-The molecular weight: by of electrophoresis SDS-PAGE.

2.4 Method of calculation and data processing

To determine the results of optimization experiments the influence of these factors on the objective function, response surface methodology RSM (Response Surface Method) and 5.0 Mode software was used to process the results. Response surface method is the method effective in maximizing the food processing process. Here is a collection ofmathematical modeling techniques and statistics, the association between the processing of data and establishment of regression equations to describe the input parameters to the nature of the product. In particular, quadratic mixed plans turn of mind instead of quadratic planning does notrotate when determining the coefficient of regression equations. To plan the mixture isrotated, the value of the weights α from conditions: $\alpha = 2\ k/4$ (when people plan to 2 k). The score at the Center plan no be increased to no matrix degenerate, so the plan will avoid the error when specifying Y in the laboratory of surface performance may be lower than in the calculations get according to the equation of regression. Experimental models made according to

mathematical models of software 5.0 Modde, during experiments, methods of optimization of the four elements. In particular, thevalue "0", "-1" and "1" the values in mind and the editor of the review of experimental planning experiments. Value of α, α-"is determined based on the lever rule. X1, X2, X3, X4 is the optimum required variables. During the experiment, the value of Q2 and R2 indicates the reliability of the modelexperiments, R2 is a real variable, and the variable rate is Q2. In particular, R2 > 0.8 and0.5 and misleading > Q2 between ∈ [0.2; 0.3] shows the regression value that is meaningful and credible model.

By the method of orthogonal four factors, regression equations are represented as follows:

Y = b0 + b1x1 + b2x2 + b3x3 + b4x4 + b12x1x2 + b 13x1x3 + b14x1x4 + b23x2x3 +b24x2x4 + b34x3x4 + b11x12 + b22x22 + b33x32 + b44x42

The results of the regression equations generated from the equation Modde 5.0 is the only variable coding (when value p < 0.05) were transferred to real variables in the following ways:

$$x_j = \frac{Z_j - x_j^o}{\Delta x_j} \quad j=1,2,...k$$

$$\Delta x_j = \frac{x_j^{max} - x_j^{min}}{2} \qquad x_j^o = \frac{x_j^{max} + x_j^{min}}{2}$$

Z_j: actual value of factors (variables).

x_j: encoding values of factors (variables).

X_0: base level value.

3. RESULTS AND DISCUSSION

3.1 Optimization of enzyme activity, pH, temperature and time of hydrolysis by experimental planning method

From the results of the experiments, the optimization conducted by the method of orthogonal experiment planning four elements: enzyme activity, pH, temperature and time (see table 1). The structure has a mind with the objective function is the performance recovery of soluble protein (Y). Core values are determined from experiments on enzyme activity: 27.828 UI/g raw beans, pH 6.5, 50^{o}C temperature, time of 120 minutes (see table 2). In the process of planning the experiment took place at the same time change the elements surveyed to establish rules that the influence of these factors to the objective function. On the basis of which to pick out the optimal parameters. Optimal testing number in this process is N = 31 experiments, including 7 experiments in mind in order to increase the level of accuracy of the process.

Optimizing experimental results are presented in table 2.

Solve planning experiments for the objective function by Mode 5.0 to the method-level Center of rotation, the results are presented in table 3.

Data in the table 3 shows the variables all have affected the objective function (Y) by P < 0.05. However, there are a few variables with P > 0.05 as X_3, X_1X_2, X_1X_3, X_1X_4, X_2X_3, X_2X_4, X_3X_4. In which the values of the enzyme content (X_1), pH (X_2), time (X_4) in tier 1 has a positive influence, while that temperature (X_3) in step 1 have a negative influence on the performance of dissolved protein recovery. From data in table 3, regression equation expressing the relationship of the variables in the process of hydrolysis of the following:

$$Y = 52.3457 + 1.2100\ X_1 + 1.0983\ X_2 + 1.2358\ X_4 - 1.4762\ X_1^2 - 3.1362\ X_2^2 - 2.8462\ X_3^2 - 1.2900\ X_4^2$$

Regression equation with real variables are converted as follows:

$$Y = -841.8808 + 2.8696Z_1 + 165.2801Z_2 + 11.3202Z_3 + 0.3852Z_4 - 0.0477Z_1^2 - 12.5449Z_2^2 - 0.1138Z_3^2 - 0.0014Z_4^2$$

Where:

Z_1: real variables of the active enzyme hydrolysis process (UI/g raw beans)

Z_2: real variables pH parameters of hydrolysis process.

Z_3: real variables of the process temperature (^{o}C).

Z_4: real variables of the process time (minutes).

Based on the results of the Anova analysis, the results are statistically significant at P<0.05. The value in this experiment, R^2= 0.966 and Q^2 = 0.807 (see table 4) satisfy all the above conditions, shows the regression values meaningful and reliable models. Regression equations are represented in three dimensions and projection to the surface responds as shown in figure 1-6.

To earn certification, a regression equation 3 repeat experiments conducted independently of the enzyme activity, pH, temperature, processing time enzymes in table 5. Experimental results obtained in table 6.

Soluble protein recovery performance of average earnings is 52.589 ± 0.267% in line with the results of experimental planning is 52.961%. From the results of the survey process, the technology is chosen for the hydrolysis process as follows: enzyme activity is 30.05 UI/g raw beans, pH 6.6, the time is 133 minutes, temperatureis 49.8^{o}C.

3.1 Survey soy extraction process by water

Looking into table 7, when the water content increases extraction rate from 1: 4; 1: 5; 1: 6 then the

recovery performance soluble protein increased gradually from 33.422% to 39.435% and reached 43.237% in ratio of 1: 6. But when counting turn water content used from the rate 1: 6; 1: 7; 1: 8 then the recovery performance soluble protein does not increase that descending from 43.237 29.125% down; According to the analysis of Anova and LSD, in the ratio of 1: 4; 1: 5; 1: 6; 1: 7; 1: 8 then the recovery performance soluble protein difference statistically significant at the 95% confidence level. Bean: water in 1: 6 for performance the highest soluble protein recovery rate 1: 6 is fit to make soy protein extraction by water. Water is the protein extraction environment, so the proportion of legumes: water can affect the performance of dissolved protein recovery. When using the water content is too high, the amount of water in the mix more making the impact or the contact between the blade of the blender and the pea not radically reduce the performance quoted. Explaining that when the water content increases from passing rate: 1 water: 6 to 1: 8 then the soluble protein recovery performance. When the water content using high amounts higher beans affect the viscosity of the mixture makes the process difficult grind, and the water is not enough to tempt the molecule dissolve out causing inefficient extraction process. Explaining that when water content use declining from bean: 1 water: 6 down 1: 4 then the soluble protein extraction efficiency decreased from 43.237 33.422% down; So to find the right amount of water to soluble protein recovery performance is the highest (see figure 7).

3.3 Comparison of hydrolysis method using Flavourzyme enzymes extraction method and by water in soy protein recovery.

3.3.1 Soluble protein recovery

In table 8, with the same basic rate: water is 1: 6 but the method of hydrolysis by Flavourzyme back to recovery performance higher soluble protein with water extraction method is 9.352%. The cause of the phenomenon is due to water extraction method only involves the electronic structure is dissolved while the hydrolysis method for enzyme hydrolysis the protein will not dissolve or massive structures, the Prince has a small volume dissolved easily. At the same time this increase in soluble protein carries an important meaning increases the nutritional value of soy milk.

3.3.2 Molecule weight

Proceed to the hydrolysis soybean protein extraction and by water in conditions as above, then the room will be conducting electrophoresis analysis, the results obtained as figure 8.

Results determined the degree of hydrolysis is depicted in table 9. As for the method of hydrolysis by Flavourzyme hydrolyzed peptide containing the epidemic, there is approximately 12kDa molecules, small amounts of peptide segments in large amounts, 31kDa, 17kDa and peptide 24kDa segment. For water extraction method, the room contains soy peptide molecules of a majority in the 75-25, 150kDa-50kDa and small quantities of peptides smaller segments 20kDa. Through electrophoresis analysis results show that the process of hydrolysis of protein from soybean by Flavourzyme for low molecular peptide in the range 10-31kDa and smaller amounts is located approximately 31-38kDa. So the process of hydrolysis of soy protein dramatically reduces the molecular mass in the range 15-150kDa into molecules has a mass of about 10-31kDa. Compared study of Mo-Nan Zhang (2014) the hydrolysis of organic substance SPI (soy protein isolate) by Flavourzyme then obtained the majority of peptides having molecular mass from 2-10kDa in DH = 6.79%[11]. Such molecular analysis of results and the level of hydrolysis are consistent. Such a method of hydrolysis by the enzyme increases the nutritional value of soy milk because the peptides are small volume easily soluble and easy to digest, take advantage of sources of protein in soy seeds ingredients, reduce protein loss disposing in bagasse in comparison with water extraction method.

4. CONCLUSION

Parameters of hydrolysis process suitable for thus are as follow: the enzyme activity 30.05 UI/g raw beans, pH 6.6, temperature 49.8^{o}C, time 133-minutes. When it dissolves protein recovery performance reached 52.589% higher 9.352% compared to water extraction method. Molecular weight of approximately 31kDa hydrolysis during extraction method by molecular water volume is 15-150kDa. Soy milk products through hydrolysis of soluble protein and small molecule weight help the digestion and absorb easily. The product is not worried with the same status, such as soy milk and traditional extraction.

Table 1
Parameters optimization experiments

Parameter	-α	-1	0	+1	+α
X_1(enzyme activity, UI/g raw beans)	16.696	22.262	27.828	33.394	38.960
X_2 (pH)	5.5	6.0	6.5	7.0	7.5
X_3 (temperature, ^oC)	40	45	50	55	60
X_4 (time, minute)	60	90	120	150	180

Where: α = 2
X_1: active enzymatic hydrolysis processes (UI/g raw beans). X_2: pH of hydrolysis process.
X_3: the temperature of the process of hydrolysis (^oC). X_4: time of hydrolysis process (minutes).
Y: the objective function of the process of hydrolysis-soluble protein recovery efficiency (%)

Table 2
Results of optimization experiments

Enzyme activity, X_1 (UI/g raw bean)	pH, X_2	Temperature, X_3 (^oC)	Time, X_4 (minutes)	Soluble protein recovery, Y (%)
22.262	6.0	45	90	40217
33.394	6.0	45	90	41.126
22.262	7.0	45	90	42.310
33.394	7.0	45	90	45.898
22.262	6.0	55	90	39.853
33.394	6.0	55	90	40.756
22.262	7.0	55	90	42.021
33.394	7.0	55	90	43.589
22.262	6.0	45	150	44.048
33.394	6.0	45	150	44.344
22.262	7.0	45	150	43.958
33.394	7.0	45	150	46.470
22.262	6.0	55	150	42.027
33.394	6.0	55	150	44.459
22.262	7.0	55	150	45.718
33.394	7.0	55	150	45.631
16.696	6.5	50	120	42.853
38.960	6.5	50	120	51.307
27.828	5.5	50	120	38.537
27.828	7.5	50	120	42.338
27.828	6.5	40	120	42.456
27.828	6.5	60	120	40.739
27.828	6.5	50	60	45.630
27.828	6.5	50	180	50.015
27.828	6.5	50	120	52.202
27.828	6.5	50	120	52.363
27.828	6.5	50	120	52.364
27.828	6.5	50	120	52.510
27.828	6.5	50	120	52.341
27.828	6.5	50	120	52523
27.828	6.5	50	120	52.130

Table 3
Effects of independent variables to the soluble protein recovery performance

Factor	Coefficient of regression equations	The standard error	P	Conf, int(±)
Constant	52.3457	0.4346	4.26E-25	0.9213
X_1	1.2100	0.2347	9.58E-05	0.4976
X_2	1.0983	0.2347	2.51E-04	0.4976
X_3	-0.3233	0.2347	0.187297	0.4976
X_4	1.2358	0.2347	7.69E-05	0.4976
X_1*X_1	-1.4762	0.2150	3.80E-06	0.4558
X_2*X_2	-3.1362	0.2150	1.16E-10	0.4558
X_3*X_3	-2.8462	0.2150	4.91E-10	0.4558
X_4*X_4	-1.2900	0.2150	1.85E-05	0.4558
X_1*X_2	0.1900	0.2875	0.518034	0.6094
X_1*X_3	-0.1550	0.2875	0.597172	0.6094
X_1*X_4	-0.1150	0.2875	0.694415	0.6094
X_2*X_3	0.0600	0.2875	0.837292	0.6094
X_2*X_4	-0.3100	0.2875	0.296843	0.6094
X_3*X_4	0.1475	0.2875	0.614881	0.6094

Table 4
Results analysis of variance Anova of optimization experiments

Soluble protein recovery (%)	**DF**	**SS**	**MS**	**F**	**p**	**SD**
Total	31	65009.6000	2097.0800			
Constant	1	64382.7000	64382.7000			
Total Corrected	30	626.9690	20.8990			4.5715
Regression	14	605.8150	43.2725	32.7294	0.0000	6.5782
Residual	16	21.1541	1.3221			1.1498
Lack of Fit	10	21.0285	2.1029	100.4790	0.0000	1.4501
Pure Error	6	0.1256	0.0209			0.1447
N = 31		Q^2 =0.807		Cond. no. =4.6857		
DF = 16		R^2 =0.966		Y-miss =0		
		R^2 Adj. = 0.937		RSD =1.1498		

Where R_2: coefficient of determination; SS: sum of squares; DF: degrees of freedom; MS: the average squared (mean square); F: F-value. The value of F is the reliability of 95%.

Table 5
Optimal values under the experimental planning

Optimal parameter	Value
Enzyme activity (UI/g raw bean)	30.053
pH	6.583
Temperature (^oC)	49.751
Time (minutes)	133.009
Soluble protein recovery (%)	52.961

Table 6
Results of empirical experiments with optimal parameters

Experiment	Soluble protein recovery (%)	Average soluble protein recovery (%)
1	52.726	52.589 ± 0.267
2	52.281	
3	52.759	

Table 7
Effect of bean:water ratio on soluble protein recovery

Bean:water ratio	Soluble protein recovery (%)	Average soluble protein recovery (%)
1:4	33.215	$(33.422 \pm 0.229)^a$
	33.384	
	33.668	
1:5	39.684	$(39.435 \pm 0.267)^b$
	39.469	
	39.153	
1:6	**43.246**	**$(43.237 \pm 0.341)^c$**
	43.573	
	42.890	
1:7	39.604	$(39.336 \pm 0.232)^d$
	39.201	
	39.203	
1:8	29.328	$(29.125 \pm 0.190)^e$
	29.096	
	28.951	

Table 8
Compare the performance of soluble protein retrieval method of hydrolysis and extraction methods by water

Method	Average soluble protein recovery (%)	Experimental parameters
Hydrolysis by Flavourzyme enzyme	52.589 ± 0.267	- Substrate:water ratio is 1:6 - Enzyme activity: 30.053 UI/g raw bean - pH: 6.583 - Temperature: 49.751^oC - Time: 133.009 minutes
Extraction by water	43.237± 0.341	- Bean:water ratio is 1:6

Table 9
The results determine the degree of hydrolysis of soy milk

V_{NaOH} (mL)	1/α	C_{NaOH} (N)	M_p (g)	h_{tot} (mevq/g protein)	DH (%)
2.2	5	0.1	1.9455	7.8	7.8

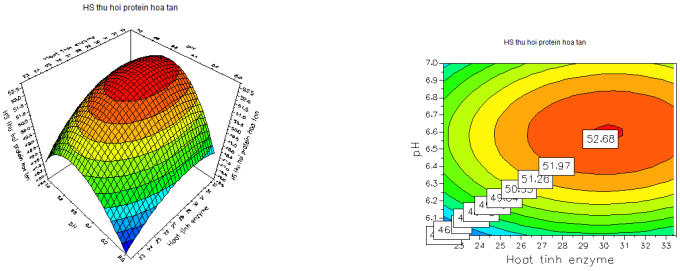

Figure 1
The effects of pH and enzyme activity and soluble protein recovery performance in three dimensions

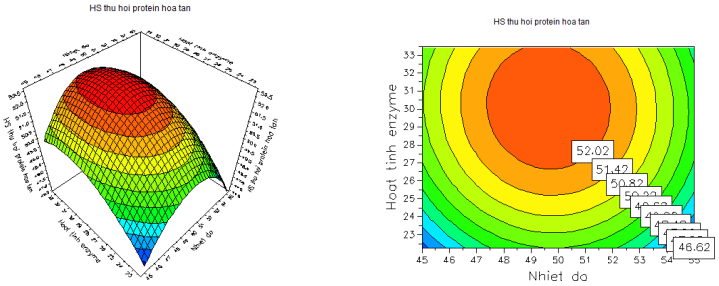

Figure 2
Effects of enzyme activity and heat recovery performance to soluble protein in three dimensions

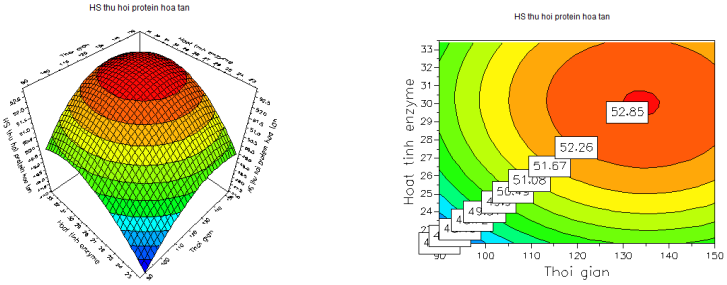

Figure 3
Effect of enzyme activity and time to recovery performance soluble protein in three dimensions

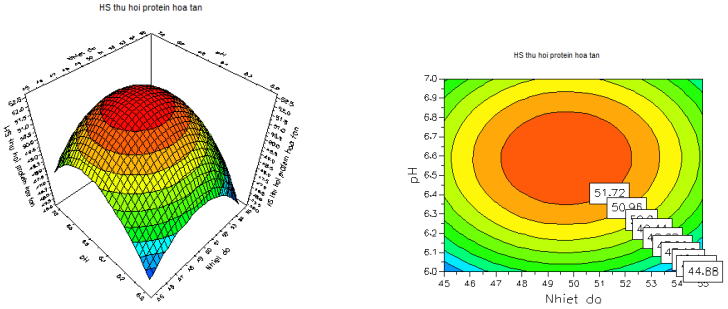

Figure 4
Effects of pH and temperature on performance recovery of soluble protein in three dimensions

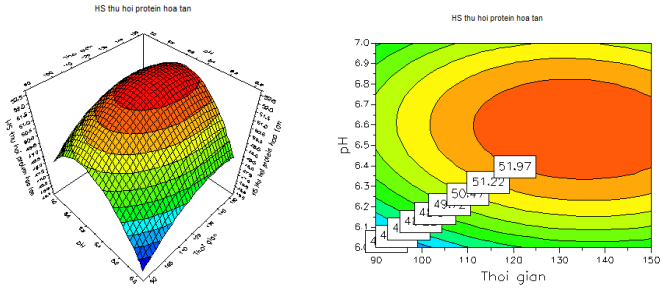

Figure 5
Effects of pH and enzyme processing time to performance recovery of soluble protein in three dimensions

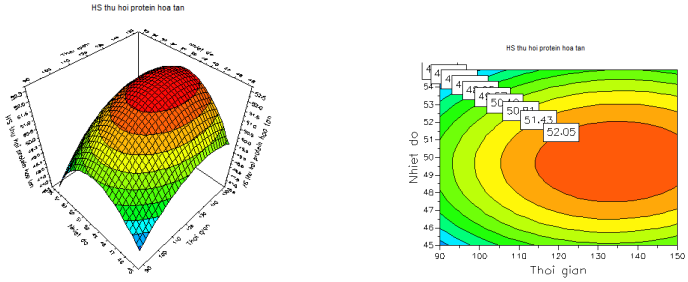

Figure 6
Effect of temperature and processing time enzymes to dissolve the protein retrieval performance in three dimensions

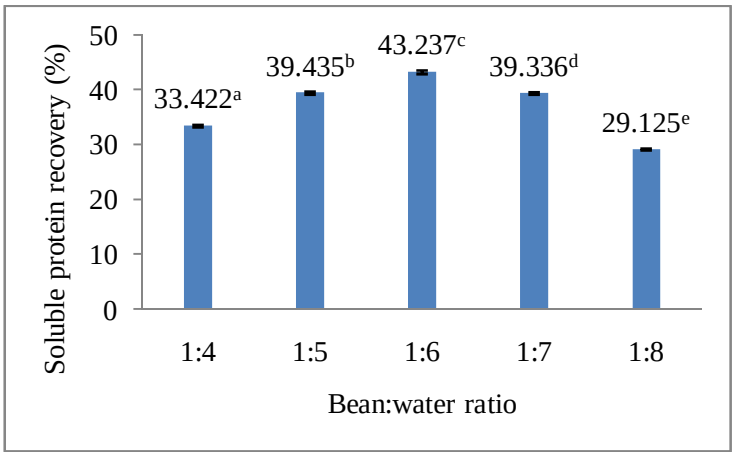

Figure 7
Effect of bean: water ratio to soluble protein recovery (%)

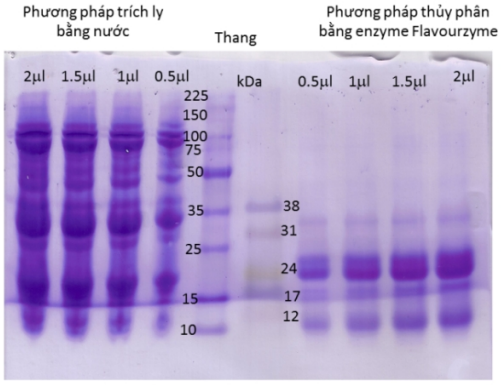

Figure 8
Electrophoresis results of soy milk by the method of hydrolysis by enzymes Flavourzyme and water extraction method

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