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Optimization of proteolytic hydrolysis from raw lean pork meat by enzymatic method to produce functional powder

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Abstract
In this study, we conducted in optimal conditions proteolytic lean pork ingredients with enzymes to produce meat protein powder products used as food for feeding patients through catheters. In particular we examine and select suitable for enzyme hydrolysis process; survey of conditions favorable for enzymatic hydrolysis is selected. Alcalase enzyme is chosen and favorable hydrolysis conditions are: fluid substrate/water ratio: 1/6; percentage of E/S (enzymes, raw materials) 3.0% (w/w); pH 7.5; temperatures of 55 ^{o}C; and the execution time is 180 minutes. The optimization process is done after already know almost exactly the value of the parameters in the process of hydrolysis of above. The result of the optimum process as follows: pH 7.49; temperature of 54.7 ^{o}C; E/S rate of 3.54% (w/w) and the duration were 197 minutes. Hydrolysis with optimum conditions the room hydrolysis to achieve the lowest viscosity 2.240 ± 0.092 cP and the degree of hydrolysis (DH) highest 31.390 ± 0.138%. The product of the hydrolysis process will be conducted by electrophoresis to determine the molecular size proteins. Then hydrolysis fluid is also performed under spray drying to reduce moisture content. The final product will be examined as part of the nutrition, amino acid concentrations, as well as biological indicators. The results showed a product of high nutritional content, easy to digest and satisfactory hygienic safety for the user.

Keywords: proteolytic, lean pork, hydrolysis, alcalase, functional powder, spray drying

Introduction
The meat, a source of nutrition and energy for people especially the meat the warm-blooded like pork, beef, poultry etc is the perfect food source which contains the amino acid does not replace and is a source of vitamin D (hard to find in plants) that helps absorb calcium, phosphorus in the body. Meat animal food sources are generally devoid of high nutrition food is classified as a group I, concentrate more nutrients essential for needs of power supply, micro chemicals, as well as the food easy processing under various forms of the dish so it is a common food in the diets of people which hardly a food would replace. Basis to our departure we chose the pork ingredients are: source material is abundant and inexpensive, stable; Ingredients: protein content in raw materials is quite high; The tradition of people's soup and consumer practices of the Vietnamese people; Work flow simple, short processing time. Mechanically complex and low production costs; highly feasible in terms of the application in the conditions of Vietnam.
Some other popular protein sources are milk, fish and eggs. Eggs contain high-protein. However, egg whites are more of cholesterol that this substance is not present in the patient. As for the material source is fish also encountered some problems, its protein content is not stable, so the difficulty of shipping and storage (in the belly of the fish has a lot of microorganisms).
Protease is the enzyme-catalyzed hydrolysis of the group associated peptide (-CO-NH-), is the primary link protein and polypeptide. Protease is capable of hydrolysis of proteins into amino acids, short peptid, and pepton. Protease has vital importance for entire biological, accounting for nearly 2% of the total number of genes encoding [3]. Proteases was studied from early eighteenth century around the end and so far it has been pretty full on studies of molecular structure as well as the application of several proteases as papain, trypsin, chymotrypsin, subtilisin [2].
Alcalase an endopeptidase proteolytic is produced by fermentation of deep surface of Bacillus licheniformis selective bacterial strains. Alcalase has been studied and identified as one of the

most effective types of bacterial proteases to produce the protein hydrolysis (Adler & Nissen, 1986; Morrisey & Benjakul, 1997; Kristinsson & Rasco, 2000; Bhaskar et al., 2007). Alcalase works optimally in some pretty wide, the temperature between 50-70 ^{o}C, and pH range 6.5 – 8.5 depending on the conditions of the particular substance. Durable wide pH range Alcalase from 5 to 11, this helps reduce the possibility of infection with microorganisms in the process of hydrolysis (Chabeaud & Associates, 2009). According to Adler-Nissen (1986), hydrolysis products with alcalase will have the highest protein content compared to the use of the enzyme papain or neutrase. A further advantage of using alcalase enzyme as catalyst for the process of hydrolysis is the bitterness of the hydrolysis products are also less than when using papain (Hoyle and Merritt, 1994). The price turn, high hydrolysis activity is also one of the advantages when using alcalase as a catalyst. Alcalase disabled totally when heat treatment at 85 ^{o}C for 20 minutes [20].

Flavourzyme is commercial protease produced from Aspergillus oryzae strain selectively without genetic mutations and used for hydrolysis of protein under the condition of neutral or slightly acid. Flavourzyme is a mixture of peptidase, medium-sized, endopeptidase and exopeptidase. It is possible to over 70% of hydrolysis of peptide linkages. The optimal temperature for about flavourzyme is 50 ^{o}C, pH about 5.0-7.0; pH for optimum operational exopeptidase is 7.0. Flavourzyme is disabled at 85 ^{o}C during 5 minutes or in 5-second time 120 ^{o}C. Solid preparations, preserved at 5 ^{o}C products can maintain activity for at least 2 months [22].

Trypsin present in pancreatic juice of humans and animals. Kiihne discovered and extracted in 1848. In 1930, Northrop and Kunitz trypsin and trypsinogen obtained in crystalline form. In body organism, trypsin activity in the alkaline environment of the intestines and serves as further hydrolysis the protein of food remaining after partial hydrolysis was in the stomach. In the pancreas, trypsin exists in the form of pre-activity is trypsinogen [2]. Trypsin is polypeptide chain containing amino acid 249, molecular weight 22680-23400 skin. The appropriate pH activity is 7.8-9.5, pHopt is 8.0. The proper temperature is 30-40 ^{o}C, optimally at 37 ^{o}C. When heated the solution to 90 ^{o}C, pH about 6-8 does not work completely. The metals in Ca, Co, Mn has the effect of active trypsin, but Cu, Ag, Hg has inhibit [2].

In the eighties of the twentieth century, it was familiar with the methods of feeding patients through catheters. Heavy patients typically are nurtured through intravenous lines to provide nutrients such as sugars, fat, protein, vitamins, mineral salts and water, etc. The aim is to improve the health status of the patient, provide nutrients, increase resistance, the ability to recover the tissue injury, rehabilitation agencies, etc. for children have to ensure growth and normal development [5]. But this approach has many disadvantages, especially if nurtured through intravenous lines completely. One of the biggest disadvantages of nurturing through the vein is completely empty gut enabling the phenomenon of bacterial infection (bacterial translocation) occurs, cause infections, blood poisoning. So, from the late 90's of the last century, researchers have developed methods of research brought up through the intestinal tract by feeding through the catheters to solve problems on [1]. Eating through the catheter is a method of raising heavy patients in most hospital due to the advantages such as fit with physiology, maintain digestive tube lining fences enhance immune function, reduce the rate of infection and contribute to shorten the duration of treatment in rehabilitation medicine for patients [5].

Purpose of our research is to optimize proteolytic hydrolysis from raw lean pork meat by enzymatic method to produce nutritional powder. After getting the optimal parameters of enzymatic hydrolysis, we evaluate molecular mass of the hydrolyzing fluid; nutrients, acid amins and microorganism in proteolytic powder so that we can give out a nutritional and safety approach for patients.

2. Material and Methods

2.1 Raw material

Fresh pork purchased from Vissan limited liability company - branch in Go Vap district HCM City, Vietnam. Use butcher's experiment is located at the back of the pig as they have a low lipid content of high protein, facilitates the process of processing. Alcalase and flavourzyme are commercially produced from Danish Novozyme and distributed by the company Nam Giang, address in 133/11, Ho Van Hue, Ward 9, Phu Nhuan istrict, HCM city, Vietnam. Trypsin: from pig pancreas, type IX-S T0303, Sigma-Aldrich and distributed by Vina Chemos agent co., Ltd. in 45 Nguyen Thi Huynh, Ward 8, Phu Nhuan district, HCM city, Vietnam.

2.2 Research method

- Survey enzyme activity and select the appropriate enzymes for hydrolysis process of meat: flavourzyme, trypsin and alcalase.
- Compare the hydrolyzing capability of flavourzyme, trypsin and alcalase.
- Survey factors affect to hydrolysis such as substrate/water (w/v), enzyme/substrate (v/w), pH, temperature, and time to viscosity and degree of hydrolyzing (DH); optimize the hydrolization.
- Determine nutrients in the hydrolized proteolytic powder: protein, acid amin, total lipid, moisture, vitamin B1 in optimal conditions.
- Determine microorganism in the hydrolized proteolytic powder.

2.3 Analytical methods

- Enzyme activity: Anson method
- Viscosity: Brookfield viscosity meter
- Degree of hydrolization (DH): (numbers of broken peptide linkage/ total numbers of peptide linkage) x 100% [18]
- Molecular mass: electrophoresis (SDS – PAGE)
- TPC: TCVN 7928 : 2008
- Coliforms: TCVN 6848 : 2007
- E.coli: TCVN 7924 – 3 : 2008
- Staphylococcus aureus: TCVN 4830 – 3 : 2005
- Clostridium perfringens: TCVN 4991 : 2005
- Listeria monocytogons: TCVN 7700 – 1 : 2007
- Salmonella: TCVN 4829: 2005

2.4 Data analysis

All experiments were repeated at least 3 times. Experimental results presented in the research are the average value of the choruses. 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 from these experiments [10]. 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 (Response Surface Method) and 5.0 Modde software

to analyze the results. Response surface method is the method effective in maximizing the food processing process. Here is a collection of mathematical 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 [10].

In particular, we used the quadratic mixed plans turn of mind instead of quadratic planning does not rotate when determining the coefficient of regression equations. To plan the mixture is turn of mind, the value of the weights α from the condition:

$\alpha = 2k/4$ (planning core 2k)

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 Modde.

Enzyme activity is determined according to the Anson method improvements. Calibration curve and and data processing are presented through figure 1. Calibration curve: y = 1.078X, with R2 = 0.999

Whereas: y: concentration of standard tyrosine (µmol/ml), x: optical density (OD)

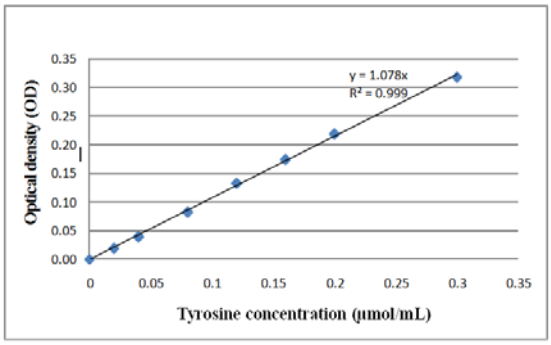

Fig 1: Calibration curve of Tyrosine

3. Results and Discussion

3.1 Determine enzyme activity

Table 1: Enzyme activity: alcalase, flavourzyme and trypsin

	Enzyme	Optical density	Equivalent mol of tyrosin (µmol/ml)	Dilution	Activity	Unit
Beginning	Flavourzyme	0.172	0.1391	2000	222.64	UI/mg
	Alcalase	0.123	0.1019	20000	1630.83	UI/ml
	Trypsin	0.281	0.2462	40000	7878.64	UI/mg
After 4 months	Flavourzyme	0.167	0.1346	2000	215.32	UI/mg
	Alcalase	0.122	0.1000	20000	1599.67	UI/ml
	Trypsin	0.281	0.2458	40000	7865.78	UI/mg

Enzyme activity of three kinds after 4 months used (flavourzyme 215.32 UI/mg, alcalase 1599.67 UI/ml and trypsin 7865.78 UI/mg) is lower than the new enzyme activity of (flavourzyme 222.64 UI/mg/ml, alcalase 1630.83 UI/ml and trypsin 7878.64 UI/mg) (see table 1). This shows that the enzyme's activity has diminished in the process of preservation and use so identified and checked to make sure the results of experiments hydrolysis of reliable. However the overall change of insignificant activity, without prejudice to the results of experiments. In the course of large-scale production, manufacturers need to focus on the change because it could reduce the likelihood of enzyme hydrolysis. Need to have reasonable preservation mode as ensuring proper temperature, divided by enzymes into the smaller volume unit, avoid light shining directly or contact the air regularly can also reduce the activity of the enzyme.

3.2 Hydrolyzing capability of different enzymes

The various enzymes gave the possibility of different hydrolysis. We surveyed 3 enzymes: flavourzyme, alcalase and trypsin. Their activity with rules about is 32, 62 UI/ml, then the concentration of turn is 14.6%, 2% and 0.4120%.

Table 2: Effect of different enzymes to viscosity and DH of hydrolyzing fluid

Enzyme	Viscosity (cP)	Degree of hydrolization
Alcalase	3.377 ± 0.150^{a}	24.658 ± 0.298^{c}
Flavourzyme	4.417 ± 0.139^{c}	10.034 ± 0.112^{a}
Trypsin	3.823 ± 0.074^{b}	19.188 ± 0.183^{b}

Three types of enzymes used in 2 groups of enzymes:

- Group 1: commercial enzymes include alcalase and flavourzyme. This enzyme group is manufactured according to industrial scale by microbial fermentation. Group of this enzyme has been shown to be capable of very good protein hydrolysis [6, 7, 8, 16, 20]
- Group 2: digestive enzymes in the body, these are groups of enzymes are extracted and purified from the body of animals. This group represents the enzyme trypsin. Trypsin, chymotrypsin and carboxypolypeptidase are the protein digestive enzymes in the body, can hydrolize 80% protein to form the dipeptide and tripeptide, amino acid [2].

With the result the hydrolysis shows, reviews of commercial enzyme groups are on the same catalytic activity, alcalase enzymes demonstrate better meat protein hydrolysis than flavourzyme lots, reduce fluid viscosity hydrolysis to 3.377 ± 0.150 cP, degree of hydrolysis (DH) 24.658 ± 0.298 the highest percent (see table 2). That's because the original meat raw materials is the polypeptide chain long circuit, alcalase is an endopeptidase cut peptide bonds on the inside the polypeptide chain. So, it's likely the hydrolysis of the meat faster, reducing the viscosity of the hydrolysis and increase the degree of hydrolysis of a lot better than just the moderate flavourzyme cutting veins, cut between the veins.

When comparing the two groups of enzymes, digestive enzymes and commercial alcalase hydrolysis demonstrate better than trypsin hydrolysis because of alcalase lower viscosity and degree of hydrolysis of achieving higher. Nowadays, when evaluating the ability to digest the in vitro evaluation based on the ability of hydrolysis enzymes trypsin [11, 15, 19]. So when alcalase demonstrates better hydrolysis than trypsin we can conclude that when hydrolysis by alcalase then proteins are cut into the polypeptide vessels small enough to

the body easily digested and absorbed. So, we decided to choose the alcalase enzyme hydrolysis process to make meat and continue to the next study.

3.3 Effect of different technical parameters to the hydrolization

3.3.1 Effect of substrate/water ratio (w/v)

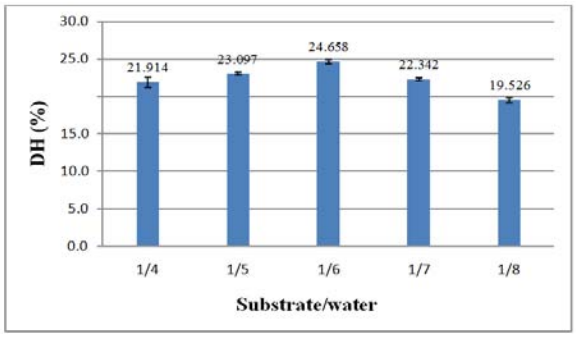

Fig 2: Effect of substrate/water ratio to DH value of hydrolizing fluid.

A number of studies by hydrolysis enzymes on many different substances were confirmed: only at a rate of organic substances, the appropriate countries, achieving the highest degree of hydrolysis of [12, 13, 17]. From figure 2, the process of meat protein hydrolysis by alcalase enzyme carried in the substrate/water ratio as 1/4, 1/5, 1/6, 1/7 and 1/8 show us when rate increases substrate/water, the degree of hydrolysis (DH) increase. Specifically, when increasing the concentration of organic substances from 125 to 166.6 g/L, DH rose from 19.526 to 24.658%. However, when increased the concentration of organic substances from 166.6 to 250 g/L, the DH did not increase further and tended to decrease. The phenomenon can be explained as follows: If the concentration of the substance is too high (200 and 250 g/L), the viscosity of the high, hard meat dispersed in water. This leads to difficult to reach peptide links of enzyme with conform so DH is not high. If the concentration of the substance is too low (142.8 and 125 g/L) concentration of the enzyme is diluted in water, the probability of the enzyme associated with the low-quality enzyme should not hydrolysis of peptide bonds are all suitable. This makes DH decreases as rate increases substrate/water. So the ratio of substrate/water works best for the process of hydrolysis is 1/6 (w/v) will be used for subsequent experiments.

3.3.2 Effect of enzyme concentration (Enzyme/Substrate, E/S)

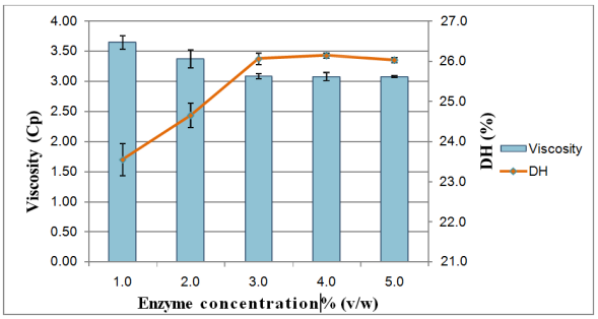

Fig 3: Effect of E/S to viscosity and DH of hydrolyzing fluid

Many studies of proteolytic enzyme concentrations of survey used to pick out suitable enzyme concentrations the hydrolysis is the highest amount of enzyme use is lowest [8, 20]. The results in figure 3 shows the increasing concentration of the enzyme hydrolysis process efficiency gained and expressed by the viscosity reduction and hydrolysis fluid level of hydrolysis increasing. Specifically, when it increased the rate of E/S from 1.0 to 3.0%, the viscosity decreased from 3.650±0.114 cP to 3.090 ± 0.044cP, while DH grew from 23.558±0.403 to 26.065% ±0.137%. However, when it increased the rate of E/S from 3.0 to 5.0%, the viscosity is not reduced and DH did not increase further and reach equilibrium. As a result, the Anova handles ascending concentrations from 3.0 to 5.0% no difference in terms of statistical significance. This is explained by the increase of enzyme preparations, the possibility of contacts between enzymes and organic matter more, the enzyme hydrolysis peptid links can easily and quickly make the viscosity decreases and the DH rose rapidly. However, with the same amount of organic substance, when enzyme concentration increases to a certain level, all fit with the peptide bonds catalyzed by enzymes have been cut entirely. Meanwhile, the process of hydrolysis products back into enzymatic inhibitory agents [9, 21]. Therefore, the equilibrium to increasing enzyme use only increased costs for the production process and the product that cannot increase the effectiveness of the hydrolysis. Therefore, the ratio E/S, the best use is 3.0% (corresponding to activity 48.92 UI/g) has effective hydrolysis of high economic efficiency.

3.3.3 Effect of pH in hydrolization

Each enzyme has a pH value max likes to suit every kind of organic matter, pH affects the level of ionized organic substrates, enzymes and affects the durability of protein enzymes. Moreover, pH also affects the dissociation of functional groups made enzyme activity centre, which essence leads to catalytic efficiency varies depending on the pH value.

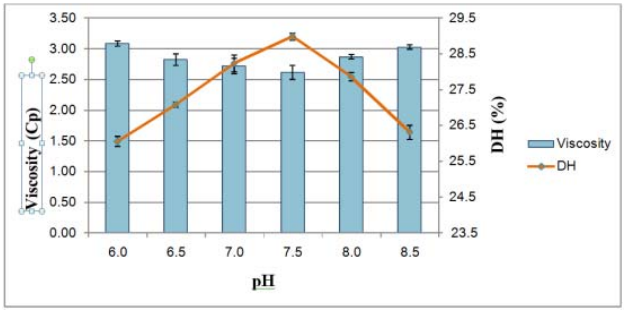

Fig 4: Effect of pH to viscosity and DH of hydrolyzing fluid

From figure 4, we survey the pH influence to the process of hydrolysis. When the pH increases, the viscosity decreases and the DH is increased. When the pH increased from 6.0 to 7.5, the viscosity decreases from 3.090 ± 0.044 cP to 2.623 ± 0.116 cP and DH rose from 26.065 ± 0.137% to 28.987 ± 0.095%. However, when further increased pH from 7.5 to 8.5, the viscosity increases and the DH value also fell. According to handle viscosity values, then Anova and DH are all expressing the difference is statistically significant, so the pH is 7.5 was chosen for the next experiment. Research results are compatible with the reality, because according to its Novozyme disclosure, the optimum pH of alcalase ranges 6.5 – 8.5 depending on the substrate. One enzyme with the different substrates, the optimum pH is also different. In

particular when the salmon skin is the optimal value is 8.39 [34], in respect of the substrate is the blood of fish Pangasius, the optimal value is 7.05 [4], pH optimum of pH is 9.45 when the hydrolysis by-product Pangasius [8].

3.3.4 Effect of temperature in hydrolization

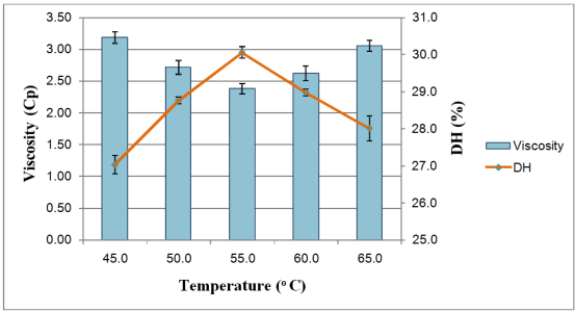

Fig 5: Effect of temperature to viscosity and DH of hydrolyzing fluid

Temperature is the major factor affecting enzyme activity and each protease activity of optimization at a certain temperature value to match the physical body. The temperature directly affects the hydrolysis process. From figure 5, surveys the influence temperature hydrolysis process to show me when temperature increases, the viscosity decreases and the DH is increased. When the temperature rose from 45 to 55 ºC the viscosity decreases from 3.187 ± 0.090 cP to 2.383 ± 0.083 cP and DH rose from 27.040 ± 0.249 percent to 30.064% ±0.155. However, when further temperature increase from 55 to 65 ºC, increased viscosity and DH value also fell. According to handle viscosity values, then Anova and DH are all expressing the difference is statistically significant, so the selected temperature value for the next experiment is 55 ºC. When the temperature increase in enzyme activity will increase the effect is due to the hydrolysis reaction increases, the molecules move more aggressively so that enzymes have many opportunities to come into contact with the substance and cut the peptide bonds in line. However, nature of the enzymes is protein that should easily be denatured at high temperatures and at which hydrolysis performance will decrease. Durability with the temperature depends on the status of existence of enzymes. The more pure enzyme, the more unreliable, the more diluted and more durable then the fluid is poor [2]. Optimum temperature of enzymes depends heavily on the origin of the substrate and the nature of it catalyzed enzyme. Optimum temperature of alcalase in blood of basa fish 60 ºC, salmon's skin 55.30 ºC, of fish byproduct is 55 ºC.

3.3.5 Effect of hydrolyzing duration

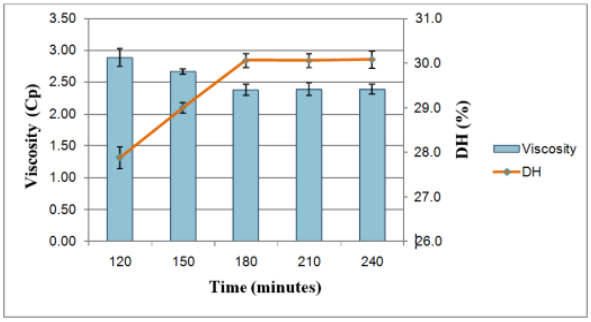

Fig 6: Effect of hydrolyzing duration to viscosity and DH of hydrolyzing fluid

The hydrolysis process requires time to process the enzyme cut the links fit, so while hydrolysis is one factor being studied extensively to control the reaction hydrolysis. Results from figure 6 shows the hydrolysis process efficiency increased rapidly during the beginning and through the viscosity reduction and hydrolysis fluid level of hydrolysis increases. Specifically, when the hydrolysis time increased from 120 to 180 minutes, the viscosity decreased from 2.893 ± 0.143 cP to 2.383 ± 0.083 cP, while DH rose from 27.890 % ± 0.245 to 30.064% ± 0.155. However, when during the hydrolysis from 180 to 240 minutes, the viscosity was not reduced and DH did not increase further and reach equilibrium. As a result of handling Anova increasing time after 180 minutes not expressed statistically significant differences. The hydrolysis process requires time to process the enzyme cut the links. The longer time the number of links that were cut as much as for the viscosity of the product reduces hydrolysis. However, up to a certain time limit, the hydrolysis process would be negligible. Should I opt for hydrolysis time is 180 seconds. The cause of the phenomenon is explained as follows: the process of hydrolysis occurred rapidly in the early stages, when a large number of peptide bonds in line be cut. The hydrolysis speed then gradually reduced and stopped when the peptide bonds are prone to hydrolysis at least gradually. The presences of the short peptides also do inhibit the enzyme hydrolysis of effects. These products function as is the nature of competing effectively with the molecules not hydrolysis or partial hydrolysis [21]. Therefore if extended too long hydrolysis DH will not increase, the viscosity will not decrease more that also affect the quality of products after hydrolysis, especially at high risk of infection with microorganisms or other unwanted side effects.

3.3.6 Optimization of hydrolization

After examining the influence of each factor: enzyme concentration, pH, temperature hydrolysis and enzymatic processing time, we continue to survey the influence at the time of the four factors to viscosity and degree of hydrolysis of DH of the hydrolysis of the fatty meat split by alcalase enzyme. We implemented optimized four elements concentration of enzyme preparations (Z1), pH is hydrolysis fluid (Z2), the temperature of hydrolysis (Z3) and processing time (Z4) by using the orthogonal model level 2 with the objective function the viscosity of the product; hydrolysis (Y1) and the degree of hydrolysis of DH (Y2). Construction of the experimental rotational variant matrix quadratic with k = 4, no = 7, conducted 31 experiments. The variables X1, X2, X3, X4 are the encoding of the Z1, Z2, Z3, Z4. Arm value α = 2 (see table 3).

With respect to the objective function is the viscosity:

The influence of each variable in the regression function is shown in table 4 with a 95% significance level. The value of X 2, X1X2, X1X3, X1X4 and X2X3 have the P > 0.05 should the effects of these variables are not statistically meaningful to value Y1. Table 4 shows the variable coefficient R2 of the regression model is variable and the value virtual 0.966 Q2 is 0.806. In experimental models of reliable value when variable valuemust be greater than Q2 virtual 0.5 and R2 in the confidence interval 0.80-0.97; When the value of R2 and Q2 as close to 1 then the regression function as a good description of the experimental results [5]. So our results are a perfect fit when conducting the planning software experimental Modde 5.0.

From table 4, we obtained regression equation expressing the relationship of the variables in the process of hydrolysis of affect the viscosity as follows:

Y1 = 2.3903 – 0.1235X1 – 0.0375X3 – 0.1291X4 + 0.1275X12 + 0.1856X22 + 0.1479X32 + 0.1133X42 + 0.0493X2X4 + 0.0922 X3X4

To find the regression equation with real variables affect the viscosity we need real variables converted under the conversion formula and the results are as follows:

Y1 = 79.3523 – 0.8885Z1 – 11.7276Z2 – 0.7689Z3 – 0.1081Z4 + 0.1275Z12 + 0.7424Z22 + 0.0059Z32 + 0.0001Z42 + 0.0033Z2Z4 + 0.0006Z3Z4

Table 3: Levels of effectiveness

Factor	Levels					Range of fluctuation λ
	-α (-2)	Lower level -1	Basic level 0	Higher level +1	+ α (+2)	
Z1[%(v/w)]	1.0	2.0	3.0	4.0	5.0	1.0
Z2	6.5	7.0	7.5	8.0	8.5	0.5
Z3[oC]	45	50	55	60	65	5.0
Z4[minutes]	120	150	180	210	240	30

Table 4: Effect of independent variables to viscosity of hydrolyzing fluid

Factor	Value of regression equation	Standard deviation	P	Conf. int(±)
Constant	2.3903	0.030079	3.27E-22	0.063764
X1	-0.1235	0.016245	1.06E-06	0.034437
X2	-0.0159	0.016245	0.342982	0.034437
X3	-0.0375	0.016245	0.034837	0.034437
X4	-0.1291	0.016245	6.03E-07	0.034437
X_1^2	0.1275	0.014882	2.25E-07	0.031548
X_2^2	0.1856	0.014882	1.17E-09	0.031548
X_3^2	0.1479	0.014882	2.99E-08	0.031548
X_4^2	0.1133	0.014882	1.05E-06	0.031548
X1X2	-0.0418	0.019895	0.05178	0.042176
X1X3	0.0108	0.019895	0.594296	0.042176
X1X4	0.0189	0.019895	0.355324	0.042176
X2X3	-0.0283	0.019895	0.173924	0.042176
X2X4	0.0493	0.019895	0.024714	0.042176
X3X4	0.0922	0.019895	0.000276	0.042176
N = 31	Q2=	0.806	Cond. No. =	4.6857
DF = 16	R2=	0.966	Y-miss =	0
	R2 Adj. =	0.937	RSD =	0.0796
			Conf.lev. =	0.95

With respect to the objective function is the degree of hydrolysis of DH

The influence of each variable in the regression function is shown in table 5 with 95% significance level. The values X3, X1X3, X1X4, X2X3, X2X4 and X3X4 have the P > 0.05 should the effects of these variables are not statistically meaningful to Y2 values. The variable X2, X12, X32, X22 and X42 have negative influences to the value Y2; Meanwhile the variables X1, X4, X1X2 have a positive influence to the value Y2. Table5 shows the variation coefficient R2 of the regression model is 0.975 and value the virtual variations Q2 are 0.858. So our results are a perfect fit when conducting the planning software experimental Modde 5.0.

From the figures above, we obtained regression equation expressing the relationship of the variables in the process of hydrolysis affect DH as follows:

Y2 = 30.0057 + 0.8643X1 – 0.1736X2 + 0.6337X4 – 0.6755X12 + 0,8959X22 - 0.8208X32 – 0.5513X42 + 0.2186X1X2

To find the regression equation with real variables affect the DH we need real variables converted under the conversion formula and the results are as follows:

Y2 = – 290.7690 + 1.6383Z1 + 52.0952Z2 + 3.6115Z3 + 0.2410Z4 – 0.6755Z12

– 3.5836Z22 – 0.0328Z32 – 0.0006Z42 + 0.4372Z1Z2

Regression equations are represented in three dimensions and response surface model (see figure 7-12).

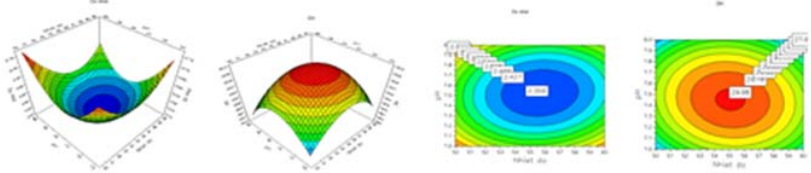

Fig 7: Effect of temperature, pH to viscosity, DH of hydrolyzing fluid in 3-D

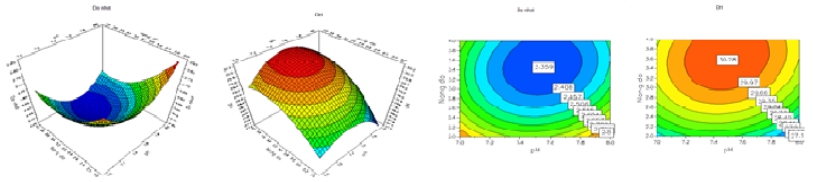

Fig 8: Effect of enzyme concentration (E/S), pH to viscosity, DH of hydrolyzing fluid in 3-D

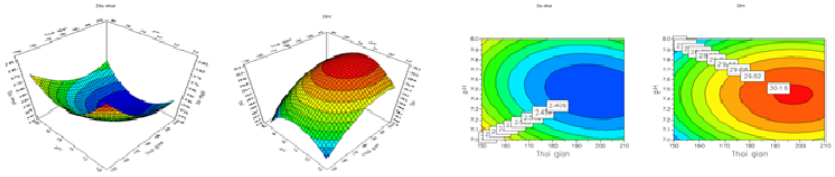

Fig 9: Effect of time and pH to viscosity, DH of hydrolyzing fluid in 3-D

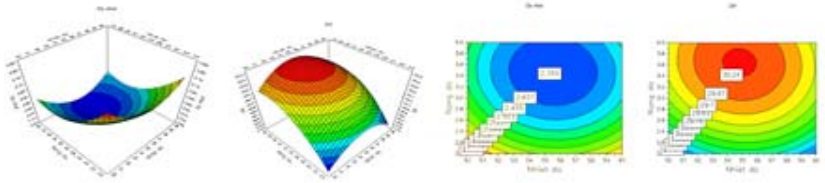

Fig 10: Effect of temperature and enzyme concentration (E/S) to viscosity and DH of hydrolyzing fluid in 3-D

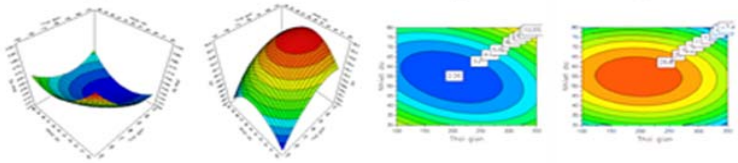

Fig 11: Effect of temperature, time to viscosity, DH of hydrolyzing fluid in 3-D

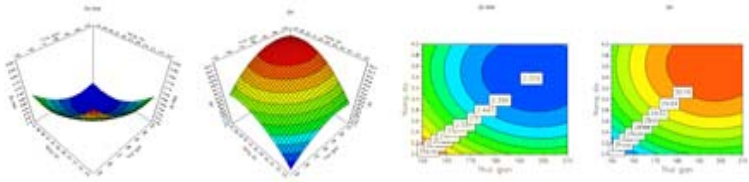

Fig 12: Effect of enzyme concentration (E/S) and time to viscosity and DH of hydrolyzing fluid in 3-D

Table 5: Effect of independent variables to DH of hydrolyzing fluid

Factor	Value of regression equation	Standard deviation	P	Conf. int(±)
Constant	30.0057	0.133523	1.99E-29	0.283057
X_1	0.8643	0.072111	2.09E-09	0.152868
X_2	-0.1736	0.072111	0.028475	0.152868
X_3	0.0021	0.072111	*0.976875*	0.152868
X_4	0.6337	0.072111	1.61E-07	0.152868
X_12	-0.6755	0.066063	2.01E-08	0.140046
X_22	-0.8959	0.066063	3.43E-10	0.140046
X_32	-0.8208	0.066063	1.24E-09	0.140046
X_42	-0.5513	0.066063	3.19E-07	0.140046
X1X2	0.2186	0.088317	0.024907	0.187224
X1X3	-0.1027	0.088317	*0.262001*	0.187224
X1X4	0.0019	0.088317	*0.982745*	0.187224
X2X3	-0.1422	0.088317	*0.126959*	0.187224
X2X4	-0.0096	0.088317	*0.915137*	0.187224
X3X4	-0.0988	0.088317	*0.279723*	0.187224
N = 31	Q2 =	0.858	Cond. No. =	4.6857
DF = 16	R2 =	0.975	Y-miss =	0
	R2 Adj. =	0.954	RSD =	0.3533
			Conf.lev. =	0.95

Optimal results by regression equations are as follows: enzyme concentration 3.5439 (%w/w); pH 7.4983; hydrolysis temperature 54.6592oC; hydrolysis time 197.034 minutes. The viscosity of the hydrolysis of the prediction of regression is 2.3292 cP and DH is 30.4625%. We conduct empirical test of the effectiveness of the process of hydrolysis of optimum process parameters. The results obtained after repeating 3 times and average the hydrolysis conditions are as follows: enzyme concentration 3.54 (%v/w); pH 7.49; temperature hydrolysis 54.7 oC; hydrolysis time 197 minutes.

The results hydrolysis fluid viscosity is 2.240 ± 0.092 cP (3.83 % false predictions); DH hit 31.390 ± 0.138% (skewed 3.04 % predicted) (see table 6). Experimental values so close to the value predicted by the equation of regression. Optimal results by regression equations are as follows: enzyme concentration 3.5439 (%w/w); pH 7.4983; hydrolysis temperature 54.592 ^{o}C; hydrolysis time 197.034 minutes. The viscosity of the hydrolysis of the prediction of regression is 2.3292 cP and DH is 30.4625%. We conduct empirical test of the effectiveness of the process of hydrolysis of optimum process parameters. The results obtained after repeating 3 times and average the hydrolysis conditions are as follows: enzyme concentration 3.54 (% v/w); pH 7.49; temperature hydrolysis 54.7 ^{o}C; hydrolysis time 197 minutes. The results hydrolysis fluid viscosity is 2.240 ± 0.092cP (3.83 percent false predictions); DH hit 31.390 ± 0.138% (skewed 3.04 percent predicted) (see table 6). Experimental values so close to the value predicted by the equation of regression.

Table 6: Comparision of optimal data by the traditional and experimental planning for pork meat

Parameter	Traditional method	Experimental planning
Concentration (%v/w)	3.0	3.54
Ph	7.5	7.49
Temperature (oC)	55	54.7
Time (minutes)	180	197
Viscosity (cP)	2.383 ± 0.083	2.240 ± 0.092
DH (%)	30.064 ± 0.155	31.390 ± 0.138

When conducting the optimal experimental planning as we see the viscosity room hydrolysis according to optimal parameters reduce 6.00% and 4.41% increase compared to the DH model optimized by classical methods.

3.4 Molecular size of hydrolized protein powder

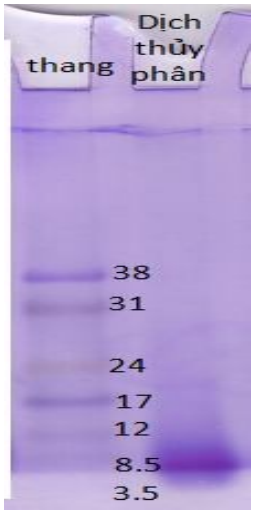

Fig 13: Electrophoresis of de-fatted hydrolized pork fluid by alcalase

According to figure 13, shows fluid of the hydrolysis of molecular sized from 3.5-8, 5 kDa, much of the focus in a size 8, 5kDa. These are shortened peptides are digested in the body easily, suitable for patients who have weak digestive tract and gastrointestinal function recovery.

3.5 Nutrient quality of hydrolized protein powder

After drying the product should be carefully avoiding packaging desiccant and identify nutrition facts, results achieved are as follows (see table 7). Products with low humidity content (below 5%) so we can extend storage time without affecting quality. This is a very good protein sources for objects to use for high protein content 90.145% (as measured by absolute dry substance). Using the product will not increase the fat content of the product because of the low fat content and fatty 0.410% (as measured by absolute dry matter). Vitamin B1 after the heat treatment, the time of hydrolysis time, exposed to the air so very low residual 0.072 (mg/100 g) should add vitamins and essential supplements in order to increase the nutritional value of the product.

Table 7: Nutrients in hydrolized protein powder by spray drying

Criteria	Quantity	Based on absolute dry matter (%)
Moisture content	4.210 %	-
Protein	86.350 %	90.145
Crude lipid	0.393 %	0.410
Vitamin B1	0.072 (mg/100g)	-

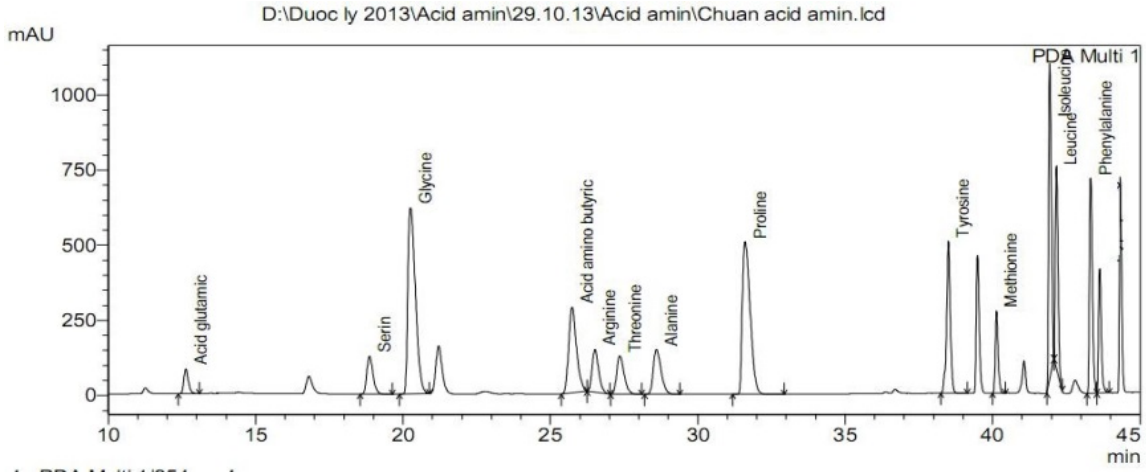

Fig 14: Chromatography of acid amin standard

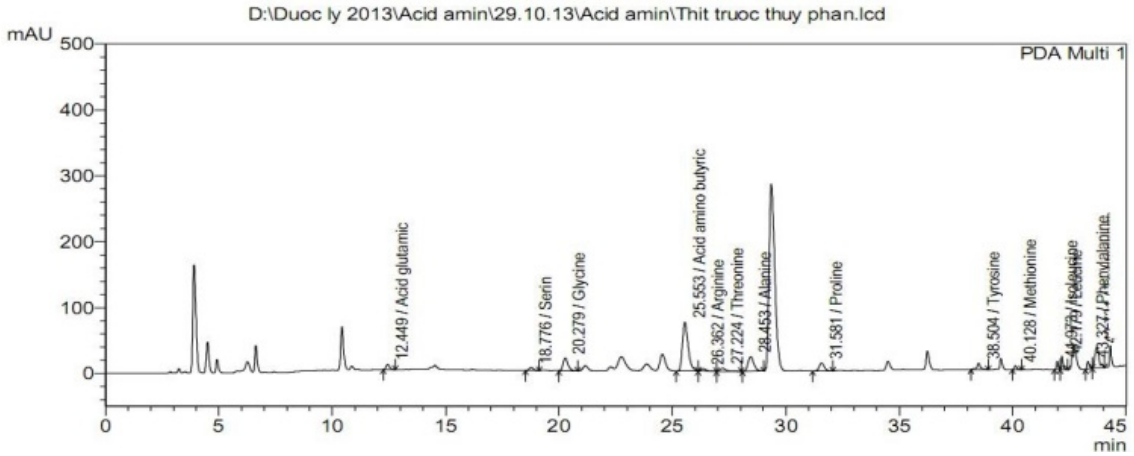

Fig 15: Chromatography of acid amin before hydrolization

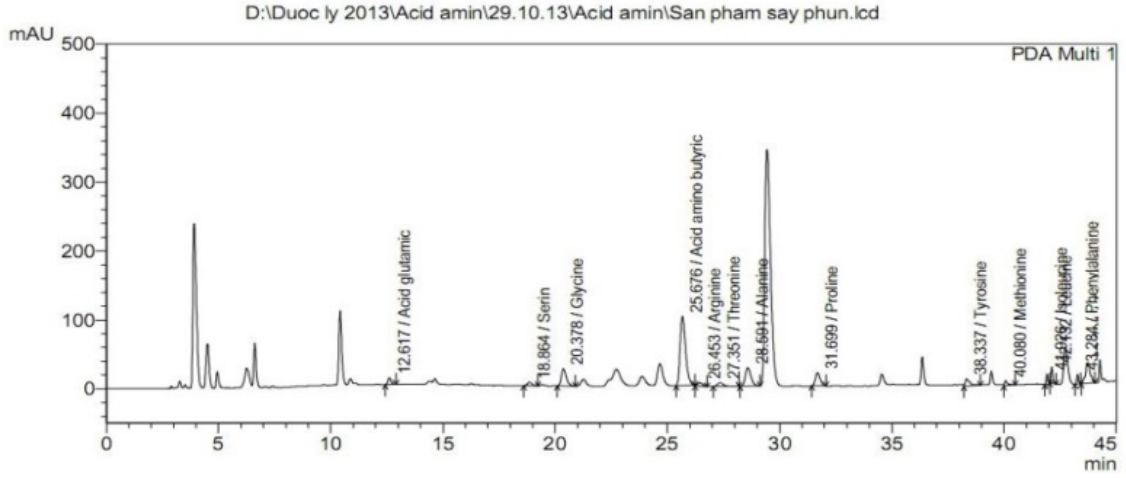

Fig 16: Chromatography of acid amin in hydrolyzed protein powder

Alcalase is an essential endopeptidase so when conducting catalyzed the hydrolysis of protein, alcalase only separated in the middle of the circuit thus reducing drastically the viscosity should hydrolysis fluid very suitable when used to produce the food flows through the catheter. However, the quantitative and qualitative amino acid in meat samples before and after hydrolysis according to table 8 and figure 14-16 show that the number and content of amino acid before and after hydrolysis does not have differences IE proteolytic by alcalase do not produce amino acid. This has also been confirmed by John b. Lloyd and Robert w. Mason, 1975 [14]. When hydrolysis by endopeptidase and the process of hydrolysis occurs most strongly, the end product can only form the tripeptide or dipeptide. With the degree of hydrolysis (DH) maximum, alcalase by hydrolysis products will survive the tripeptide, dipeptide can digest the instant when the body this assertion-protein powder products produced by hydrolysis of pork that are suitable for the patient because the response is likely to flow out of the catheter.

Table 8: Acid amin before and after hydrolization

Acid amin	Before hydrolization (mg/g)	After hydrolization (mg/g)
Acid glutamic	3.178	3.293
Serin	2.098	2.138
Glycine	2.896	2.930
Arginine	8.075	7.896
Threonine	4.243	4.285
Alanine	11.500	11.044
Proline	1.224	1.594
Tyrosine	1.064	1.124
Methionine	2.446	2.662
Isoleucine	0.705	0.695
Leucine	4.888	4.633
Phenylalanine	1.865	1.866

3.6 Microorganism in hydrolized protein powder

Table 9: Microorganism in hydrolized protein powder

Microorganism	Maximum	Value	Unit
TPC	10^3	100	CFU/g
Coliforms	50	3	CFU/g
Coliforms	50	3	CFU/g
E. coli	3	< 3	MPN/g
Staphylococcus aureus	3	< 3	MPN/g
Clostridium perfringens	10	Not detected	CFU/g
Listeria monocytogons	Not detected	Not detected	CFU/g
Salmonella	Not detected	Not detected	CFU/25g

In table 9, the product meets the standard base of the industry; meet the requirements of the standards of Vietnam on the high-nutrition product powder form, no reheating before eating.

4. Conclusion

In these nutrients are used for patients in protein and animal protein in particular is considered to be the most important source of nutrition. The study to have a common protein production process and consistently produce a product can meet the requirements of nutrition, sanitation, power flow transmission of catheters, and affordable is an urgent request at present. Thereby, it contributes to improve the quality of hospitals, protect the health of patients. Protein powder products after drying with high nutritional value (high in protein) and low fat content, meet the microbiological requirements for user safety.

5. References

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