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Enteral tube feeding nutritional protein hydrolysate production under different factors by enzymatic hydrolization

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Abstract
Hydrolysis of proteins involves the cleavage of peptide bonds to give peptides of varying sizes and amino acid composition. There are a number of types of hydrolysis; enzymatic, acid or alkali hydrolysis. Chemical hydrolysis is difficult to control and reduces the nutritional quality of products, destroying L-form amino acids and producing toxic substances such as lysino-alanine. Enzymatic hydrolysis works without destructing amino acids and by avoiding the extreme temperatures and pH levels required for chemical hydrolysis, the nutritional properties of the protein hydrolysates remain largely unaffected. In this research, we investigate the fat removal and protein hydrolization from pork meat to produce the enteral tube feeding nutritional protein hydrolysate for patient. Our results are as follows: meat moisture 75.1%, protein 22.6%, lipid 1.71%, ash 0.5%, vitamin B1 1.384 mg/100 g; n – hexan treatment at 80 ^{o}C in 45 minutes and drying 30 minutes in 90 ^{o}C. Viscosity of the hydrolysate is very low 2.240 ± 0.092 cP and high degree of hydrolization 31.390 ± 0.138 %. The final protein powder has balance nutritional components and acid amines; low microorganisms, which are safety for human consumption.

Keywords: *fat removal, protein hydrolization, protein hydrolysate, tube feeding.*

1. Introduction
Enteral nutrition is indicated for patients who have a functional GI tract, but are not able to nourish themselves by mouth. Protein hydrolysates have been defined as “mixtures of polypeptides, oligopeptides and amino acids that are manufactured from protein sources using partial hydrolysis” (Schaafsma *et al.*, 2009). There has been growing interest in these preparations over the last two decades, with novel bioactive peptides continually being discovered, as it has been shown that short-chain peptides from hydrolyzed proteins have a higher nutritive value and may be utilized more efficiently than an equivalent mixture of free amino acids (Grimble *et al.*, 1986). Milk-based products are the source of the greatest number of bioactive peptides isolated to date. Other sources include meat, eggs and fish, in addition to plant sources such as soy and wheat (Hartmann, R. *et al.*, 2007). It has emerged that protein hydrolysates have many uses in human nutrition; ingredients in energy drinks, weight-control and sports nutrition products (Frokjaer, *et al.*, 1994), sources of nutrition for elderly and immuno-compromised patients (Nagodawithana *et al.*, 2010). Clinical applications have also been suggested, including treatment of Phenylketonuria (PKU), liver disease, Crohn’s disease and ulcerative colitis (Clemente, 2000).
Production of protein hydrolysates in the food industry involves the use of digestive proteolytic enzymes from animals including chymotrypsin, trypsin and pepsin, or food grade enzymes obtained from plants and microorganisms which are regarded safe for human nutrition. Following protein hydrolysis, fractions can be categorised according to two characteristics. The first category consists of fractions with a high amino acid content. The second category consists of bioactive peptides with an amino acid sequence which is inactive in the intact protein molecule but becomes active in the hydrolysate following exposure to digestive and/or proteolytic enzymes (Aoife L. McCarthy *et al.*, 2013).
Protein hydrolysates are produced from purified protein sources by heating with acid or, preferably, addition of proteolytic enzymes, followed by purification procedures. Each protein hydrolysate is a complex mixture of peptides of different chain length together with free amino acids, which can be defined by a global value known as degree of hydrolysis (DH), which is the fraction of peptide bonds that have been cleaved in the starter protein(Grimble GK, 2000). However, even the exact information on DH cannot not tell us

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the whole story, as two protein hydrolysates made by different methods (e.g., oligopeptides/significant free amino acids *vs*. mainly dipeptides and tripeptides) may have a similar degree of hydrolysis even though their absorption kinetics are likely quite different. Consequently, all protein hydrolysates are certainly not created equal (Anssi H Manninen *et al*., 2009)

Some noteworthy researches mentioned to protein hydrolysate production can be numbered as follows: Diniz AM. (1997) optimized the recovery of nitrogen in the enzymatic hydrolysis of dogfish shark muscle by the use of response surface methodology. The optimum values for enzyme/substrate ratio, temperature and pH were found to be 3.7% (w/w), 55.3 degrees C, and 8.3, respectively. The dogfish protein hydrolysate produced under these conditions contained a high crude protein concentration (> 85%), and its high nutritional value was indicated by the presence of all essential amino acids, and by high PER values. These results indicate the potential for dogfish protein hydrolysate to be used in lieu of vegetable proteins as a protein supplement in foods.

A.M. Liceaga-Gesualdo *et al.*, (1999) examined the raw herring (*Clupea harengus*), a waste product from the roe industry hydrolyzed using an endopeptidase preparation from *Bacillus licheniformis.* Aliquots were taken at 0, 5, 10, 20, 30, 45, and 60 min hydrolysis to measure the degree of hydrolysis. The functional properties tested were emulsifying activity index (EAI), foamability, and foam stability of the hydrolysate. At 36% hydrolysis, the herring hydrolysate presented good emulsifying stability (> 120 minutes) and an adequate foam expansion (142%), as compared to the soluble fraction from the unhydrolyzed control herring. The lipid content decreased considerably to 0.77% for the fish protein hydrolysate, while its protein content increased to 77%. The amino acid composition remained similar to that of the control.

Alvaro Villanueva *et al*., (1999) produced an extensive sunflower protein hydrolysate by sequential hydrolysis with endo- and exo-proteases. A high quality protein isolate has been obtained from defatted sunflower meal by alkaline extraction and isoelectric precipitation. Protein content was increased from 31.2 % in the defatted flour to 97 % in the protein isolate. The percentages of fiber, soluble sugars, polyphenols and residual lipids in the protein isolate were reduced to more than 90 % with respect to the defatted meal. The protein isolate was used as starting material for the generation of an extensive enzymatic protein hydrolysate. The hydrolysis was carried out in a pH stat using sequentially an endo-protease (Alcalase) and an exo-protease (Flavourzyme). The protein hydrolysate, with a degree of hydrolysis of 50.7 %, was white and non bitter.

Nielsen *et al.*, (2001) established the trinitro-benzene-sulfonic acid (TNBS) method with regard to enzymatic hydrolysis. However, this method is laborious, cannot be used to follow a hydrolysis reaction continuously, and includes hazardous and unstable chemicals. This paper describes a method based on the reaction of primary amino groups with o-phthaldialdehyde (OPA). The conclusion is that the OPA method of analyzing the DH of protein hydrolyses is more accurate, is easier and faster to carry out, has a broader application range, and is environmentally safer than the TNBS method.

See S F *et al.*, (2001) produced fish protein hydrolysate from Salmon (Salmo salar) skin using Alcalase® 2.4 L. Hydrolysis conditions were optimized by using a response surface methodology (RSM). The model equation was proposed with regard to the effects of enzyme to substrate level, temperature and pH on the degree of hydrolysis. An enzyme to substrate level of 2.50% (v/w), temperature of 55.30°C and pH of 8.39 and were found to be the optimum conditions to obtain the highest degree of hydrolysis (77.03%) using Alcalase. The freeze dried protein hydrolysate was characterized with respect to chemical composition and amino acid composition. The protein hydrolysate produced contained high protein (89.53%) and higher level of indispensable amino acids. Protein hydrolysate from salmon skin may potentially serve as a good source of desirable peptide and amino acids.

Nilsang S et al, (2005) produced fish protein hydrolysate (FPH) from fish soluble concentrate (FSC), a by-product from canned fish industry, by using Flavourzyme™ and Kojizyme™. Hydrolysis conditions were optimized by using a response surface methodology (RSM). The model equations were proposed with regard to the effects of temperature (*T*), time (*t*), and enzyme concentration (*E*) on the degree of hydrolysis (DH). The optimum values for Flavourzyme™ concentration, substrate concentration, temperature, and hydrolysis time were found to be 50 LAPU/g protein, 20% (w/w), 45 °C, and 6 h, respectively (LAPU; Leucine Aminopeptidase Unit). While those values for Kojizyme™ were 40 LAPU/g protein, 20% (w/w), 50 °C, and 6 h, respectively. Kojizyme™ enhanced the formation of some bitter-taste amino acids such as tryptophan during hydrolysis process whereas Flavourzyme™ did not. The spray-dried FPH produced with Flavourzyme™ contained high protein content (66%). The bitterness of FPH was less than that of 1 ppm caffeine solution.

Souissi N. et al (2006) examined Biochemical and Functional Properties of Sardinella (*Sardinella aurita*) By-Product Hydrolysates. Fish protein hydrolysates (FPHs) with different degrees of hydrolysis (DH of 6.62, 9.31and 10.16) were prepared from heads and viscera of sardinella (*Sardinella aurita*) bytreatment with Alcalase®. The liquid hydrolysates were spray dried. All spray dried by-product hydrolysates contained 73 to 75 % of proteins and low content of lipids. Reversed-phase HPLC profiles showed that low DH hydrolysates contained high hydrophobic peptides and the extent of hydrolysis resulted in an increase of fish hydrophilic peptides. Some functional properties of FPHs were assessed and compared with those of casein or the undigested sardinella protein. Solubility increased while emulsifying capacity and whippability decreased with the increase in the degree of hydrolysis. The product, with a DH of 10.16 %, had an excellent solubility (100 %) over a pH range of 6.0–10.0. The antioxidant activity of the hydrolysates was also tested. All FPHs exhibited more than 50 % inhibition of linoleic acid peroxidation. The antioxidant activity of FPH with 10.16 % DH was about 73 % of that of a-tocopherol, a natural antioxidative agent.

Imelda Wing (2007) studied 16 protein hydrolysate samples were produced from commercial shrimp (*Pandalopsis dispar*) processing wastes using Taguchi's L16 experimental design. Four factors, namely water-to-substrate ratio, percent enzyme, time of hydrolysis and type of protease, were investigated. The properties of the shrimp waste hydrolysates were assessed by three responses: product yield, degree of hydrolysis and bitterness. It was found that the type of protease had the most significant impact on hydrolysate

properties. Hydrolysates produced from the proteases had significantly higher soluble product yields compared to the controls incubated without added protease. Moreover, the yield from treatments with Alcalase or Protamex reached over 30%, which was six times higher than the control samples. In terms of degree of hydrolysis (DH), Alcalase, Flavourzyme and Protamex gave higher DH than bromelain, which had a DH similar to the controls. Despite the high soluble yields and DH for Alcalase and Protamex hydrolysates, their bitterness was intense and the 10% (w/v) solutions of these samples were evaluated to be greater than 2000 ppm caffeine. Bromelain and Flavourzyme samples had significantly lower bitterness close to 1500 ppm caffeine but these samples were still significantly more bitter than the controls, which contained nearly no bitterness. A sample (blended in 2:1 water-to-substrate ratio and hydrolyzed with 4% Alcalase for 4 hours) that gave a soluble yield of 37.64%, a DH of 2.30 meq/g and bitterness of 2300 ppm was selected for further fractionation by size and hydrophobicity to study the characteristics of the bitter substances. Results showed that bitter substances were small having molecular weight under 3 kDa and contained a large amount of hydrophobic amino acid residues such as Tyr, Phe, Leu, Ile and Lys. Therefore, it was concluded that Flavourzyme had the best potential to be used to produce protein hydrolysates from shrimp processing discards and small hydrophobic peptides were the major contributors to the bitterness.

Jia Jianping (2010) investigated enzymatic hydrolysis of Alaska pollack (*Theragra chalcogramma*) skin and antioxidant activity of the resulting hydrolysate. The optimal hydrolysis conditions were as follows: hydrolysis time 8 h; enzyme/substrate ratio 2:1000; skin/water ratio 1:6; temperature 55 degrees C; pH 6.0. Under these conditions the highest yield of peptides was 83.44%, with 85.95% of the hydrolysate being mainly composed of oligopeptides with molecular weights ranging from 180 to 1000 Da. The hydrolysate showed 2, 2-diphenyl-1-picrylhydrazyl radical-scavenging activity, with an IC (50) value of 2.5 mg Ml (-1), and its reducing power was 0.14 at 1 mg mL (-1), 53.8% of that of reduced glutathione at the same concentration.

Amiza *et al.*, (2011). Determine the combined effects of hydrolysis time, temperature, pH and ratio of enzyme to substrate on the degree of hydrolysis (DH) of silver catfish frame using Response Surface Methodology. The proximate compositions of silver catfish frame and silver catfish hydrolysate powder were determined as well. The effects of independent factors were described using a three-level factors Face Centered Central Composite design. The suggested hydrolysis conditions for obtaining the optimum DH using Alcalase® were – temperature of 55oC, hydrolysis time of 163 min, pH of substrate at 9.45 and an enzyme concentration of 2.0%. The generated model showed a quadratic fit with experimental data. Proximate analyses revealed that silver catfish frame contained 25.02% protein, 68.21% fat and 7.08% ash. While silver catfish frame hydrolysate powder contained 65.05% protein, 32.92% fat and 0.86% ash. The protein recovery in silver catfish frame hydrolysate was as high as 71.6%.

Amiza *et al.*, (2012) determined the effect of degree of hydrolysis (DH) on the physicochemical properties of cobia frame hydrolysate. Three levels of degree of hydrolysis of cobia frame hydrolysate were studied, which were 53%, 71% and 96%. After enzymatic hydrolysis using Alcalase®, the samples were spray-dried. Cobia hydrolysate powder samples were analyzed for their proximate analysis and physicochemical properties. The proximate analysis showed significant differences in fat and ash content only. DH96 hydrolysate showed desirable essential amino acid profile for human requirement except for methionine and isoleucine. The study found that cobia frame hydrolysate had good colour, emulsifying capacity and excellent foaming properties. However, there were no significant differences in water-holding capacity, oil-holding capacity and peptide solubility among the hydrolysate samples. This study suggested that cobia frame hydrolysate is a potential ingredient and foaming agent for food industry.

Murna Muzaifa *et al.*, (2012) studied the production of fish protein hydrolysate (FPH) from fish by-product prepared by enzymatic hydrolysis. Fish by-product were prepared using Alcalase and Flavourzyme enzyme and properties of FPH were analyzed. The results showed that FPH prepared using Alcalase enzyme had greater amount of protein (82.66%) than FPH prepared using Flavourzyme enzyme (73.51%). Solubility, emulsifying and foaming properties of FPH prepared using Alcalase were also better than those prepared using Flavourzyme enzyme. The FPH derived from fish by-product using enzyme may potentially serve as a good source of protein. It could be used as an emulsifier and as a foaming agent.

Purspose of our research is to investigate different factors affecting to the enzymatic hydrolization of lean meat to produce the enteral tube feeding nutritional protein hydrolysate for patient.

2. Material & Method

2.1 Material

The meat was purchased from the Vissan Co. Ltd in HCM City, Vietnam. Alcalase and flavourzyme are commercially produced from Danish Novozyme.

2.2 Research method

Raw material quality evaluation includes protein, total lipid, moisture, ash and vitamin B1. Enzyme activity (flavourzyme, trypsin and alcalase) and the appropriate enzymes for hydrolysis are surveyed. We also examine different factors influencing the process of fat from raw materials such as solvent type, processing time and drying time needed to remove residual solvents remaining. Purpose is to define the types of solvents, while the fat content of the types of fat left in the raw materials is the lowest. And identify appropriate drying time to remove the remaining solvent. Meat is mixed with solvents according to the ratio of 1: 10 and kind of fat by following the hot extraction (i.e. temperature heat is set greater than 10oC solvent's boiling point). Use solvents: chloroform, diethyl ether, n-hexane. Treatment time with solvents is 15-75 minutes. Drying time to remove solvent is 10-50 minutes. Then we 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 Testing method

– Enzyme activity: Anson method

- Viscosity*:* Brookfield viscosity meter
- Degree of hydrolization (DH): (numbers of broken peptide linkage/ total numbers of peptide linkage) x 100%
- Molecular mass: electrophoresis (SDS – PAGE)
- Total protein TCVN 4328 – 1: 2007
- Crude fat: AOAC 920.39
- Moisture content: TCVN 4326: 2001
- Ash: TCVN 4327: 2007
- Residual solvent: GC – MS
- 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 Statistical analyses

All experiments were repeated at least 3 times. They are processed by ANOVA (Startgraphics) to check the significant difference via LSD.

3. Result & Discussion

3.1 Nutritional component in meat

Table 1: Nutrient content in raw material meat

Criteria	Content (based on wet matter %)	Content (based on dry matter %)
Moisture content	75.100	-
Protein	22.600	90.763
Crude fat	1.710	6.867
Ash	0.500	2.008
Vitamin B1	1.384 (mg/100g)	-

The meat has high moisture content (75.1%) and nutritional elements so it's easily contaminated by microbial infection. It must be careful in preservation and processing time to avoid contamination. The survey results show that lean meat have a high protein content 90.763 %, relatively high amounts of vitamin B1 1.384 (mg/100 g), So this is a source of protein and vitamin suitable for the research. The lipid (6.867%) in material should be removed during the process of hydrolysis.

3.2 Enzyme activity

Enzyme activity is determined according to the Anson method. Calibration curve 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).

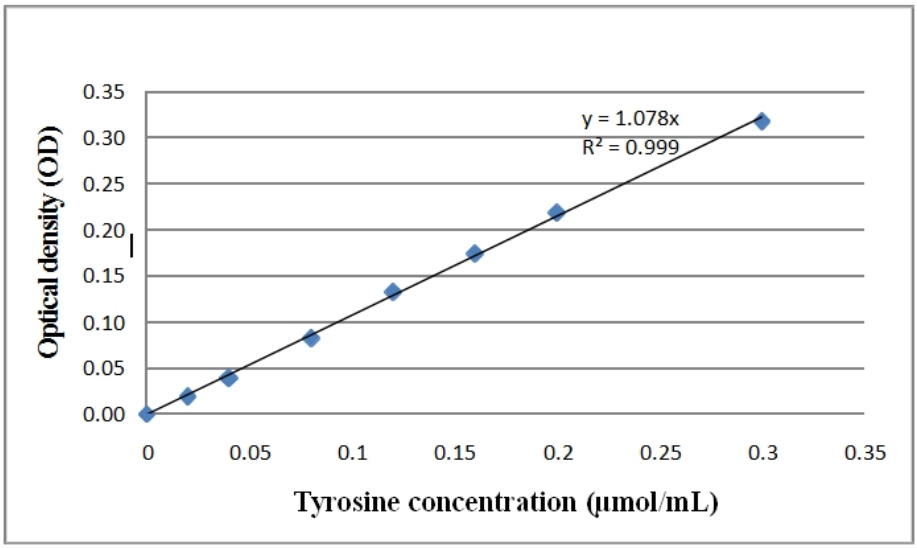

Fig 1: Calibration curve of Tyrosine

Table 2: Enzyme activity: alcalse, 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 after 4 months includes flavourzyme 215.32 UI/mg, alcalase 1599.67 UI/ml and trypsin 7865.78 UI/mg. They are lower than the new enzyme activity (flavourzyme 222.64 UI/mg/ml, alcalase 1630.83 UI/ml and trypsin 7878.64 UI/mg) (see table 2).

3.3 Different solvents for fat removal

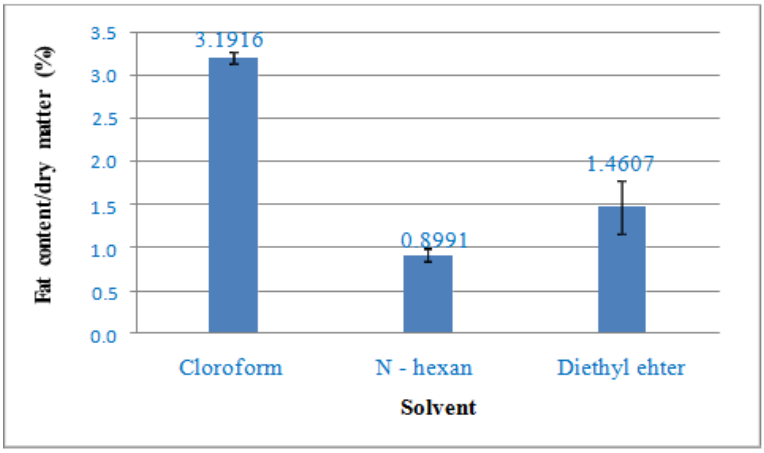

Fig 2: Fat removal by different solvents

From figure 2, n-hexane is chosen as solvent for fat removal in further researches

3.4 Treatment time of n – hexan to fat removal

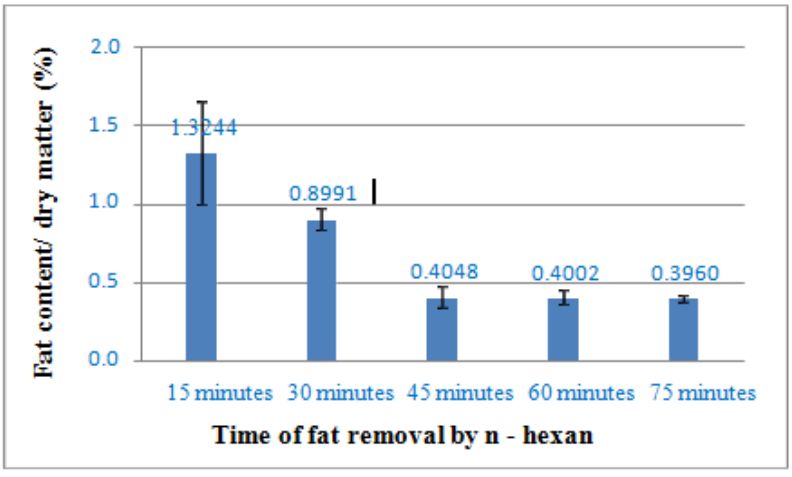

Fig 3: Treatment time of n-hexan to fat removal

Treatment time varies; the fat content in meat is also different. The longer processing time is, the more solvent exposes to meat. Our result shows that processing time is 45 minutes is adequate to reach 0.4048 ±0.0693% (see figure 3). Meanwhile, if we increase the processing time up to 60 and 75 minutes, the fat remained in meat change not much at reliability 95% compared to the treatment in 45 minutes. So, treatment time 45 minutes is adequate.

3.5 Drying time to n-hexan residual removal

The drying time to remove residual solvents is essential. Processing temperature should be higher the boiling point of the solvent in order to ensure full evaporation of the solvent. Drying time decided to the amount of solvent left in the meat. So 20 minutes is enough to remove n-hexan.

Table 3: Drying time to n – hexan residual removal

Sample	Area of n – hexan
Standard	3.39146
Drying in 10 minutes	16.98183
Drying in 20 minutes	8.10688
Drying in 30 minutes	0
Drying in 40 minutes	0
Drying in 50 minutes	0

3.6 Hydrolization by different enzymes

Table 4: Different enzymes to viscosity and DH of hydrolization

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

We decided to choose the alcalase enzyme for hydrolysis process in the next study

3.7 Various factors affecting to the hydrolization
3.7.1 Substrate/water ratio (w/v)

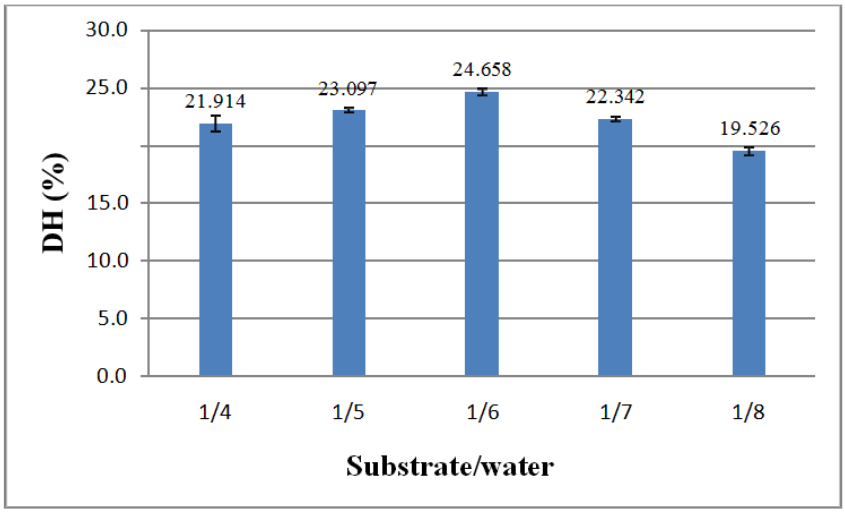

Fig 4: substrate/water to DH of hydrolization

Figure 4 shows the effect of different substrate/water ratios 1/4, 1/5, 1/6, 1/7 and 1/8 to the hydrolization. From figure 4, se see that 1/6 (w/v) is optimal for subsequent experiments.

3.7.2 Enzyme concentration (Enzyme/Substrate, E/S)

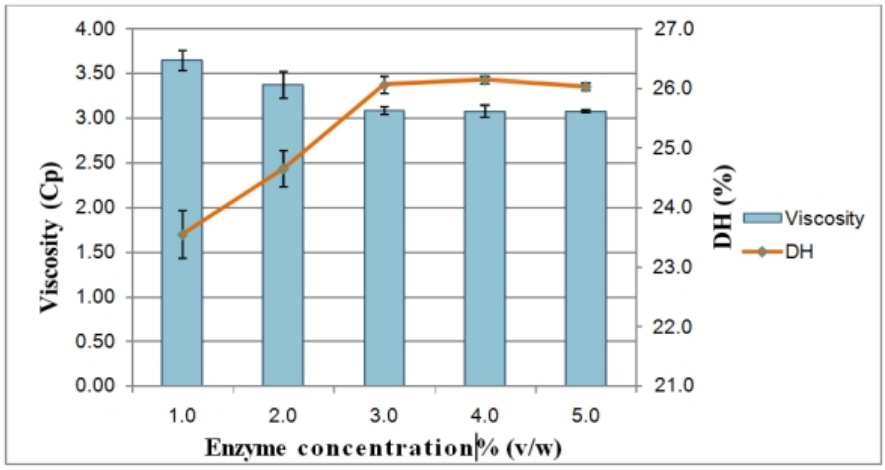

Fig 5: E/S to viscosity and DH of hydrolization

The best use is 3.0% (corresponding to activity 48.92 UI/g) has effective hydrolysis because of its high economic efficiency.

3.7.3 pH in hydrolization

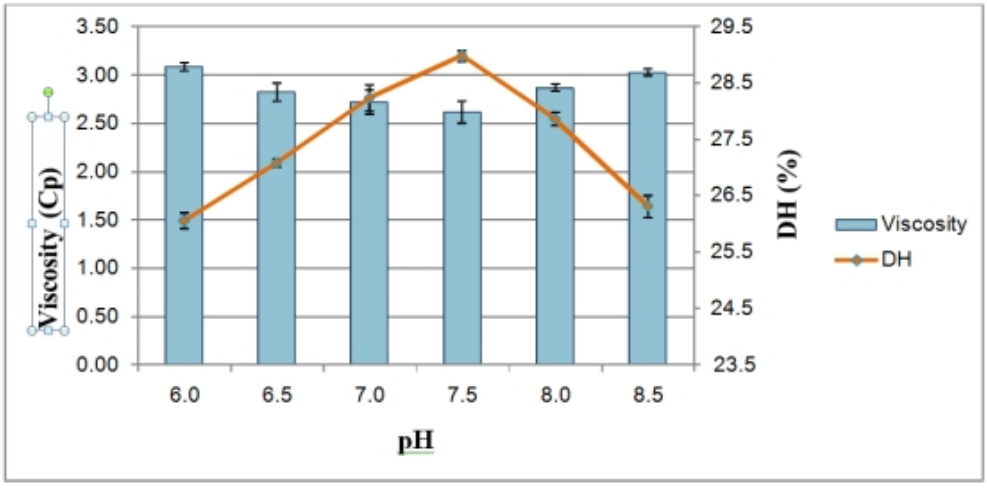

Fig 6: pH to viscosity and DH of hydrolization

Each enzyme has optimal pH value. pH affects the level of ionized organic substrate and the durability of protein. Moreover, pH also affects the dissociation of functional groups made enzyme activity center, which leads to catalytic efficiency. pH is 7.5 was chosen for the next experiment.

3.7.4 Temperature in hydrolization

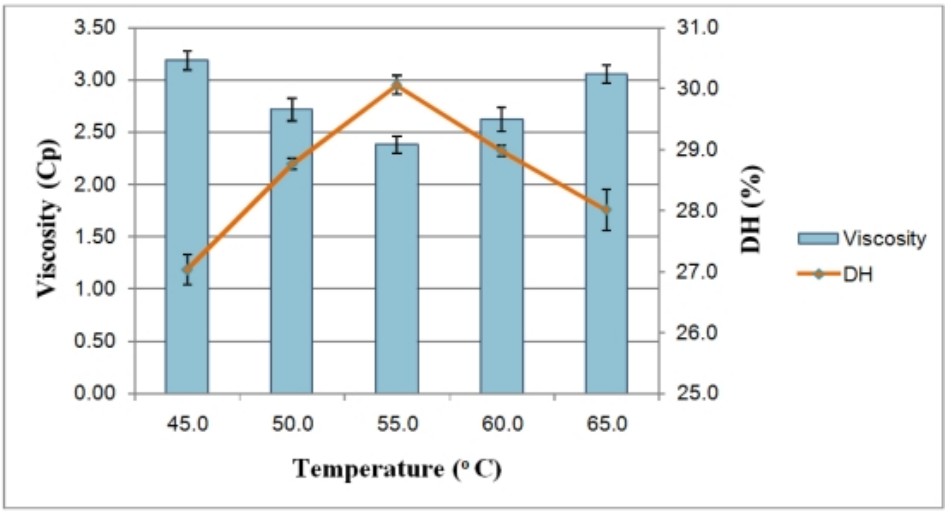

Fig 7: Temperature to viscosity and DH of hydrolysate

Temperature is the major factor affecting enzyme activity. The temperature directly affects the hydrolysis process. From figure 7, we survey the influence temperature to hydrolysis process. When temperature increases, the viscosity decreases and the DH increases. When the temperature rises from 45 to 55ºC the viscosity decreases from 3.187 ± 0.090 cP to 2.383 ± 0.083 cP and DH rises from 27.040 ± 0.249 percent to 30.064% ±0.155. However, temperature increase from 55 to 65ºC, viscosity increases and DH value also falls. When the temperature increases, enzyme activity will also increase that affecting to the hydrolysis reaction. The molecules move more aggressively so that enzymes have many opportunities to come into contact with the substance and cut the peptide bonds in. However, nature of the enzymes is protein so it is easily denatured at high temperature. Durability with the temperature depends on the status of enzyme existense. The more pure enzyme is, the more unreliable, the more diluted and the more durable of the fluid are. Optimal temperature of enzymes depends heavily on the origin of the substrate and the nature of it catalyzed core. We select 55 ºC for next experiment.

3.7.5 Hydrolization time

The hydrolysis time increases from 120 to 180 minutes, the viscosity decreases from 2.893 ± 0.143 cP to 2.383 ± 0.083 cP, while DH rises from 27.890 % ± 0.245 to 30.064% ± 0.155. However, the hydrolysis from 180 to 240 minutes, the viscosity is not reduced and DH doesn't increase further.

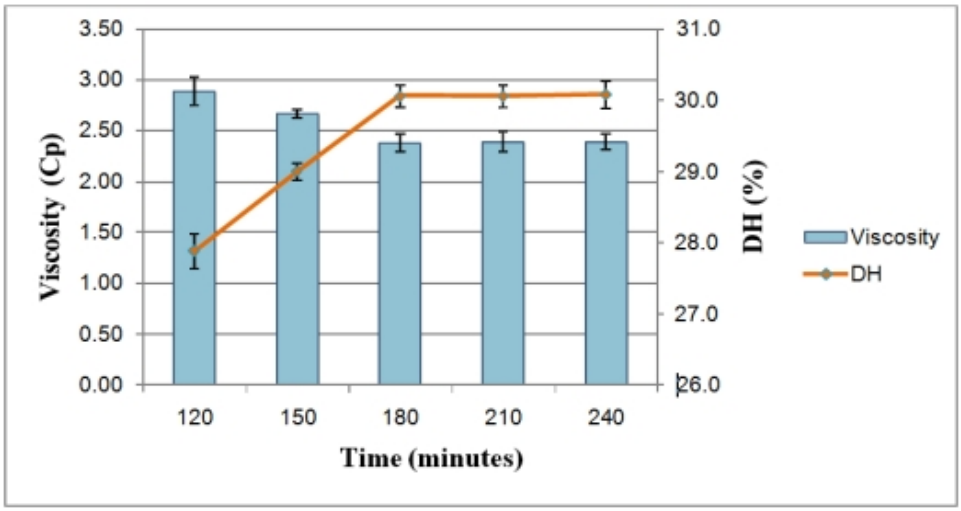

Fig 8: Hydrolization time to viscosity and DH of hydrolysate

3.7.6 Optimization of hydrolization

Table 5: Comparision of optimal data by the traditional and experimental planning

Parameter	Traditional method	Experimental planning
Concentration (%v/w)	3.0	3.54
pH	7.5	7.49
Temperature (°C)	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

3.8 Nutrient quality of protein hydrolysate

Table 6: Nutrients in protein hydrolysate 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)	-

Product has low humidity content (below 5%) so we can extend storage time without affecting to its quality. This is a very good protein sources for patient with low fat content 0.410%. However vitamin B1 lost too much after the heat treatment so it's necessary to add more vitamins and essential supplements to increase the nutritional value of the product.

Table 7: 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

3.9 Microorganism in hydrolized protein powder

Table 8: 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

The product meets the standard regulated by Vietnam Health Ministry.

4. Conclusion

Humans require a protein intake sufficient to maintain the body nitrogen balance and allow for desirable rates of deposition during growth and sickness. Ingestion of protein amounts greater than requirements results in the excess being metabolized and excreted. Conversely, in the case of inadequate dietary protein intake, the body utilizes its own proteins as a source of nitrogen; therefore a regular and sufficient intake is essential. Protein hydrolysate performs a number of key functions in the body including the building and repair of tissues, cell signaling and the provision of energy. Protein hydrolysate also performs enzymatic and structural functions.

5. Reference

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