# Production and Characterization of Spray Dried Protein Hydrolysate from Kidney Bean ( *(Phaseolus L. Vulgaris)* ) Prepared by Enzymatic Hydrolysis

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## Abstract

Protein hydrolysate from kidney bean (*Phaseolus L. vulgaris*) has been prepared by enzymatic hydrolysis process using papain enzyme. Evaluation of the extent of protein hydrolysis was conducted by measuring the degree of hydrolysis (DH). The optimization of protein hydrolysate production has been carried out by analyzing the influences of papain enzyme concentration, temperature, and time of hydrolysis on the degree of hydrolysis (DH) using RSM Design. The optimized product was spray-dried and analyzed the proximate (moisture, lipid, protein) content. The protein hydrolysate powder product was characterized the foaming capacity and stability, and also by FTIR, and DSC methods. The optimum condition of enzymatic hydrolysis of kidney bean protein was obtained by an addition 3.0 % of papain enzyme at 58$^{o}$C for 7 hours. The functional groups present in the kidney bean protein hydrolyzate are amine group, amino, carboxyl; C-O, based on the thermal test, there is a peak point of the 7S protein (vicilin) and 11S (legumin), DH of powdered protein hydrolyzate is 6.30% and the liquid hydrolysate is 7.06%. The highest foaming capacity and stability of this product was reached at pH 3.0. DSC analysis of the product showed two peaks (Tm) at 66.2$^{o}$C and 105.6$^{o}$C.

**Keyword:** Protein, Hydrolysate, *Phaseolus L. vulgaris*, papain, RSM.

## 1. Introduction

Kidney beans commonly referred to as *Phaseolus vulgaris L* are Legume types are very important in human consumption. Red beans already cultivated 70,000 years ago and plays an important role in traditional food people in various regions of the world (Thapa, 2012). Kidney beans are a type plants that are not native to Indonesia, but rather from the west southwest Mexico, South America and mainland China. Furthermore this plant spreads to other regions in the world including Indonesia. Although this plant is not native to Indonesia, but is often found in Indonesia. Many areas in Indonesia Planted with these plants are Lembang, Pacet, Batu City, and Lombok Island (Praptiningrum, 2015). Hydrolysis of protein by strong acids, strong bases or proteolytic enzymes resulting in the form of amino acids and peptides. Hydrolysis with a strong acid is nonspecific and attack all peptide bonds, produces a large number of fragments (Al- Bahri et al. 2009). Enzymatic hydrolysis of protein is the alternative process to improve its functional properties without influencing the nutrition value. The nutritional and functional properties such as solubility, foaming and emulsion stability were improved by enzymatic hydrolysis. Some studies reported the advantage of hydrolyzed protein in human health such as less allergenic, easy digested and absorbed (Kain et al. 2009). Hydrolysis of protein increased the number of peptides and the hydrophobic of amino acid residues would contribute to the formation of the emulsion. Fish protein hydrolysate products have been used as nutritional supplements (Prabha et al. 2016). The functional properties of rapeseed protein have been improved by using different enzymes. Nitrogen solubility, foaming properties, water and fat adsorption capacity of rapeseed meal are improved after hydrolysis by Ficin enzyme. Meanwhile, Oil adsorption capacity, foaming and emulsifying properties of this protein can be improved after hydrolysis with alcalase (G. Chabanon et al. 2007). The objective of this study was to produce the protein hydrolysate (FPH) from kidney beans using papain enzyme and to characterize the spray-dried of FPH product both physical and chemical properties.

## 2. Materials and Methods

### Materials

Kidney beans were purchased at a traditional market in Bandung, Indonesia. Commercial papain enzyme (brand Xian Arisun ChemParm Co. Ltd, CAS no. 2323.627-2, Shaanxi, China) in the form of powder. All chemicals used were of analytical grade.

### Preparation of protein hydrolysate (PH)

PH preparation was carried out by using a modification of Priatni, et al. (2017) and Anissa, et al. (2017) methods. The kidney bean was cleaned and mixed with distilled water with a ratio of 1:4. Samples were blended and pH adjusted to 7.0. The optimization of PH production was carried out in a water bath by using 2.0-3.0 % of papain at 40-60$^{o}$ C for 3-7 hr. The enzymatic hydrolysis process was stopped at 85$^{o}$C and allowed to stand for 15 min. PH extract was

vacuum-filtrated and the filtrate was stored at -20° C. For product characterization purposes, the filtrate sample was spray dried at 160°C (inlet) and 80°C (outlet).

**Optimization of enzymatic hydrolysis condition of PH by RSM-CCD method**

RSM-CCD (Response Surface Methodology - Central Composite Design) was used to predict the optimal hydrolysis condition for PH using papain enzyme. Optimization of enzymatic hydrolysis was used three factors i.e. the influences of enzyme concentration, temperature and hydrolysis time. Percentage of degree hydrolysis (% DH) was used as a parameter of hydrolysis. Twenty hydrolysis trials were randomly run per CCD. The center value was selected according to references which are 0.3% papain enzyme, 55°C and 5 hours (Silpradit K et al. 2010, Sathish & Murthy, 2009, Auwal et al. 2017). Design Expert 7.0 software was used in this experimental design. The optimum condition was used for PH powder production that prepared by spray-dried the supernatant of FPH with inlet temperature 160°C and outlet temperature 80°C.

**Proximate analysis and yield of PH powder**

The moisture, protein, fat and ash content of PH powder were determined according to AOAC (2002). Soluble protein content was analyzed by using a modification of Lowry method. The yield of FPH powder was calculated by using the following formula:

$$\text{yield } (\%) = \frac{W2}{W1} \text{ x } 100\%$$

W2 = mass of FPH powder
W1 = mass of eel fish fillet

**Determination of Degree Hydrolysis**

Degree hydrolysis (DH) of peptone extract was calculated using the relationship between α-amino nitrogen (AN) and total nitrogen (TN) according to equation [10]:

$$\%DH = \frac{\alpha\text{-amino nitrogen (AN)}}{\text{Total nitrogen}} x100$$

Total nitrogen was determined by Kjeldahl method. α-Amino nitrogen was analyzed using a modification of Wang et al. (2012) method. The concentration of α-AN was calculated using the following equation:

$$\alpha - AN\ (\%) = \frac{V}{W\ x\ 10}\ x\ N\ NaoH\ x\ 14.008$$

V: titration volume, W: the weight of the sample.

**Foaming capacity and stability**

The foaming capacity and stability of PH from the powder sample were determined according to Naqash and Nazeer (2013) method with modification. The foaming capacity was calculated as,

$$\text{foaming capacity } (\%) = \frac{(A - B)}{B} \text{ x } 100\ \%$$

Where,
A is the volume after whipping (ml)
B is the volume before whipping (ml)
Foam stability was calculated as follows:

$$\text{foaming stability } (\%) = \frac{(A - B)}{B} \text{ x } 100\ \%$$

**Fourier transforms infrared spectroscopy (FTIR)**

The functional group of FPH powder sample has been analyzed by FTIR (Thermo Scientific, Nicolet iS5 iD5 ATR) technique according to Rosli and Sarbon's (2015) method with modification. The FTIR spectra were obtained from discs contained FPH powder in potassium bromide (KBr). Duplicates samples were analyzed and spectra from 4000 to 550 $cm^{-1}$ were obtained at a data acquisition rate of 4 $cm^{-1}$ at room temperature. The functional group of FPH was monitored from the spectra and compared to references data. The peaks obtained from the spectra of samples were assigned based on functional groups present in the sample.

**Determination of melting temperature**

FPH powder sample melting temperature (Tm) was determined by a differential scanning calorimetry (DSC) technique (Netzsch, DSC214), according to Ren et al. (2010) method with modification. The samples were filled in a pan, weighed and pressed by a hydraulic handle. Samples were analyzed at a heating rate of 10$^{o}$C/min ranging from 20 to 12$^{o}$C. The transition midpoint (Tm, or the melting temperature) is the temperature at which half the protein molecules are folded and half are unfolded.

## 3. Results and Discussion

The optimization of the hydrolysis process of PH from kidney beans was determined according to the percentage of the degree hydrolysis (%DH). The optimization of enzymatic hydrolysis was analyzed by RSM-CCD design. Analysis of variance (ANOVA) of the response surface quadratic model for DH of PH was presented in Table 1. Overall data showed that the model had significant ($p<0.05$) with $R^2$ value was 0,8269.

Table 1. Analysis of variance (ANOVA) of degree hydrolysis PH

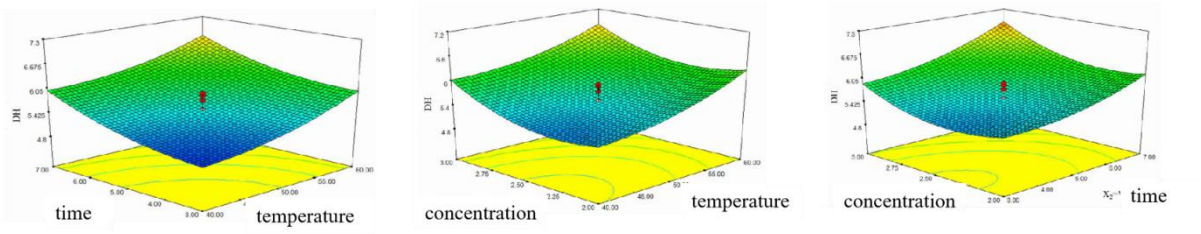
| Source | Sum of squares | df | Mean Square | FValue | p-value Prob > F | |
|---|---|---|---|---|---|---|
| Model | 9.028034 | 9 | 1.003115 | 5.30708 | 0.0077 | significant |
| $X_1$ -temp. | 2.536197 | 1 | 2.536197 | 13.41801 | 0.0044 | |
| $X_2$ -time | 2.625625 | 1 | 2.625625 | 13.89113 | 0.0039 | |
| $X_3$ -Konsentrasi | 1.300598 | 1 | 1.300598 | 6.880945 | 0.0255 | |
| $X_1$ $X_2$ | 0.016833 | 1 | 0.016833 | 0.089056 | 0.7715 | |
| $X_1$ $X_3$ | 0.016833 | 1 | 0.016833 | 0.089056 | 0.7715 | |
| $X_2$ $X_3$ | 0.046758 | 1 | 0.046758 | 0.247378 | 0.6297 | |
| Lack of Fit | 1.348375 | 5 | 0.269675 | 2.488837 | 0.1698 | not significant |
| $X_1^2$ | 0.523202 | 1 | 0.523202 | 2.768053 | 0.1271 | |
| $X_3^2$ | 1.792885 | 1 | 1.792885 | 9.485439 | 0.0116 | |
| $X_2^2$ | 0.584263 | 1 | 0.584263 | 3.091102 | 0.1092 | |
| Residual | 1.890144 | 10 | 0.189014 | | | |
| Pure Error | 0.541769 | 5 | 0.108354 | | | |
| Cor Total | 10.91818 | 19 | | | | |

Table 1 shows the ANOVA analysis of the degree of hydrolysis response using a quadratic model. Based on the data, it can be seen that the quadratic model used has a p value of 0.0077 ($P < 0.05$) so that the model used has significant value. The independent variable is used are temperature, time and concentration of the enzyme papain, these three variables has a value of 0.0044; 0.0039; 0.0255, respectively. These three variables are used has a P value <0.05 so that it has a significant value.Lack of fit from the quadratic model degree of hydrolysis of red bean protein is 0.1698 ($P > 0.05$) which means it has not significant value. This indicates that the quadratic model is suitable to be used to determine the optimum conditions for the hydrolysis of the red bean protein. The equation for DH and the response variable (Y) of PH was derived

using the regression coefficient of intercept, linear, enzyme concentration, time and quadratic terms to fit a full response surface model. The equation was given as follow:

$$Y=5.5709+0.4309X_1 +0.4385X_2 +0.3083X_3 +0.3527X_3^2$$

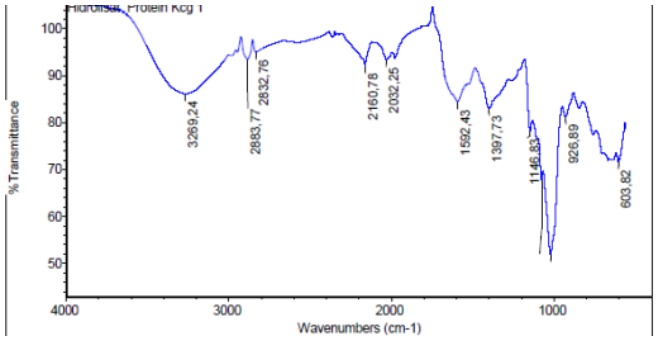
*Figure 1. 3D surface plot of interaction between temperature and time (a), temperature and concentration (b), time and concentration (c) factors on degree hydrolysis (DH) of PH from kidney beans*




a b c

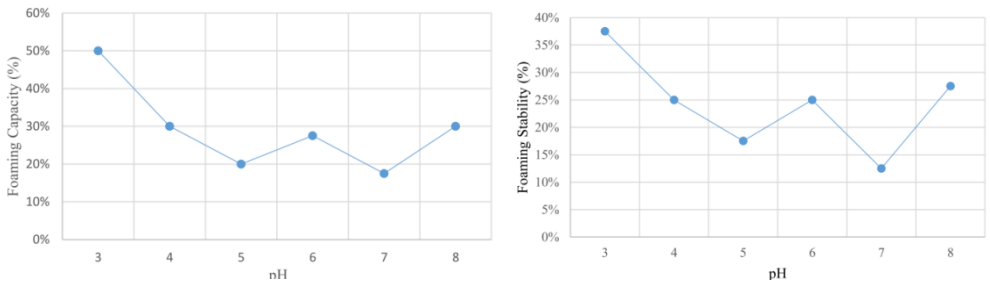
On graph 1a shows the effect of temperature and time in degree of hydrolysis. Based on the graph above, it can be seen that the maximum DH can be obtained at the reaction time the longer it is used as well as the temperature.In the contour plot analysis, DH will have a high value when using the reaction time between 6 to 7 hours and the temperature used is between 55 to 60ºC. Graph 1b shows the effect of temperature and papain enzyme concentration in the degree of hydrolysis.Plot contour analysis shows that DH will have a high value at the time using the concentration of the papain enzyme at levels 2.75% to 3% and temperature used is between 55 to 60ºC. Meanwhile, graph 1c showing the influence of time and Papain enzyme concentration in the degree of hydrolysis.based on this analysis, the degree of hydrolysis (DH) produced has a range between 4,893% to 7,217%. The optimization process using expert design software.The independent variables used are temperature, papain enzyme concentration and temperature with the percentage of DH as the response to produce the combination of model. Based on the data, the optimum condition of enzymatic hydrolysis of kidney beans protein was obtained by an addition 3.0 % of papain enzyme at 58$^o$C for 7 hours. The predicted value of the degree of hydrolysis of red bean protein is 7,284%.

*Figure 2. FTIR spectra of PH from kidney beans*



From FTIR spectra (Figure 2) was identified 9 absorption, there is 1 strong band, 2 medium bands,1 shoulder band and 5 weak bands. Strong band that is absorb at 3263.7 $cm^{-1}$; medium band i.e. absorb of 1591.55 $cm^{-1}$; 1397.66 $cm^{-1}$; 1 shoulder band is the absorption of 1146.85 $cm^{-1}$; 5 weak bands namely 2883.92 $cm^{-1}$; 2160.88 $cm^{-1}$; 2033.33 $cm^{-1}$; 665.28 $cm^{-1}$; 598.11$cm^{-1}$. The strong band in the absorption is the bond of O-H and N- H. This is because the two bonds are between 3000-3700 $cm^{-1}$. In this absorption is indicated as a group amine, this is because the amine will show clear at 3000-3700 $cm^{-1}$ and to the left of the C-H absorption. This is proven by the existence absorption 2883.92 $cm^{-1}$ which is a stretching band/CH. Absorption at 1591.55 $cm^{-1}$ represents CH-$NH_2$ or an amino group and absorption 1397.66 $cm^{-1}$ is a COOH bond or carboxyl group, this group was suggested as the amino acid. This is due to of amino acids are monomers protein consisting of amino groups and carboxyl groups. Moreover, this also proves that the hydrolysis process of in the preparation of protein hydrolysate the complex bonds of proteins can produced the amino acids (Belitz, Grosch, & Schieberle, 2009). Absorption at 1146.85 $cm^{-1}$ is a ribbon stretching / stretching C-O an at 2160.88 $cm^{-1}$ and 2033.33 $cm^{-1,}$ represent the existing bands caused by the diamond used on the FTIR. While the two absorption is 665.28 $cm^{-1}$ and 598.11 $cm^{-1}$ are the fingerprint area (Ven, et al., 2002).

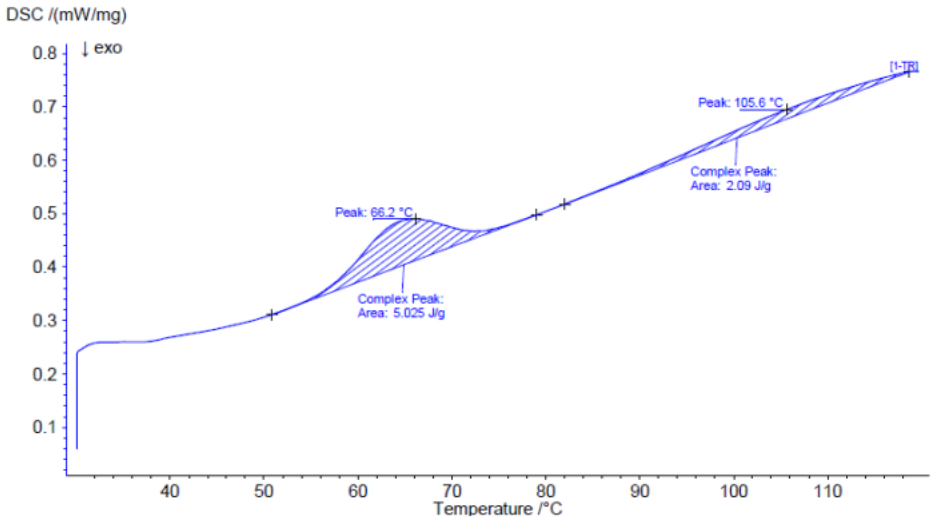
*Figure 3. Profiles of the Foaming Capacity (a) and Foaming Stability (b) at different pH values of PH from kidney beans*




The results analysis of foam capacity and foam stability are presented in Figure 3. The data shows that the highest of foam capacity (50%)and foam stability (38%)of red bean protein hydrolysate were at pH 3.Foam capacity is associated with the level of flexibility of protein. Protein will diffuses faster into the air-water interface to wrap the particles of air which will ultimately increase the foam capacity (Deng, et al., 2011).This study suggested that protein hydrolysate from kidney beans contain aspartic acid or glutamic acid due to both of these amino acids have an isoelectric point at a pH around 3 (Wade, 2006). Foam stability will have a higher value on pH close to the isoelectric point of the protein. This is due to attraction intermolecular

electrostatic resulting a maximum pH value that increasing the stiffness and thickness of protein adsorbed on interface between air and water (Kempka, Horvath, Fagundes, & Prestes, 2015).

*Figure 4. DSC thermogram of PH from kidney beans*




Differential Scanning Calorimeter (DSC) analysis is the method to analyze the denaturation temperature of food protein. This method is very important for food protein application. The results analysis of hydrolysate powder red bean protein was presented in Figure 4. the data shows there are two peaks which are 66.2ºC, 5.0H with ΔH 5,025J/g and 105.6 C and with ΔH 2.09J/g.The first denaturation temperature or peak point value of red bean PH can be related to the denaturation of 7S (vicilin) and the second peak related with 11S (legumin) globulin, which is the dominant protein in beans (Parra, 2018). ΔH is a value both exothermic and endothermic contributions due to the breakdown of hydrophobic bonds and hydrogen. This correlates with the contents of the secondary structure of the protein. The difference of ΔH value is influenced by hydrolysis time which indicated that the product will be stabilized by hydrogen bonds and hydrophobic interactions (Ortiz & Añón, 2001).

## 4. Conclusion

The outcome of this study shows the potential usage of kidney beans for the production of PH through the enzymatic hydrolysis using papain enzyme and serves as a protein supplement.

The structural analysis of the PH product found the presence of amine groups and carboxyl group related to the existence of amino acids. Foam capacity and foam stability analysis suggested that protein hydrolysate from kidney beans contain aspartic acid or glutamic acid due to both of these amino acids have an isoelectric point at a pH around 3. The denaturation temperature of red bean protein hydrolysate is related to the denaturation of 7S (vicilin) and 11S (legumin) globulin.

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