# Functional Properties of Protein Hydrolysates from Skipjack Tuna By-products Using Response Surface Methodology

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Received: 8 March 2024
Accepted: 12 December 2024
Published: 30 December 2024
Academic Editor: Dr. Tatty Yuniarti

 Accreditation Number:148/M/KPT/2020. ISSN: 2089-5690, e-ISSN: 2406-9272. https://doi.org/ 10.15578/squalen.829

## Abstract

Protein hydrolysates from skipjack tuna by-products are rich in protein and excellent in functional properties, making them a valuable source of nutrients for humans. This research sought to determine the optimal pH, temperature, and hydrolysis time for producing protein hydrolysates from skipjack tuna by-products (PHST) using Response Surface Methodology (RSM). A total of 20 g of PHST was prepared from frames and trimmings, samples were hydrolyzed under the following conditions: pH of 6 (P1) and 7 (P2), temperatures of 50°C (S1), 60°C (S2), and 70°C (S3), and hydrolysis times of 90 minutes (T1), 180 minutes (T2), and 270 minutes (T3). The hydrolysis process was terminated by inactivating the enzyme at 80°C for 30 minutes. The filtrate was ready for further analysis in the laboratory. Data and design experiments were analyzed using Box-Behnken Design (BBD) with the Design-Expert (DX) 13® software (Stat-Ease Inc. Minneapolis) to determine the optimum conditions for higher PHST production. A quadratic model was developed to predict the production of PHST. The RSM recommendation was to perform hydrolysis at pH 6.386 and a temperature of 61.190°C for a hydrolysis time of 228.540 minutes to result in a desirability of 0.906 in producing PHST with 85.680% DH, a protein solubility of 51.538%, and a viscosity of 3.587%. The study results showed that PHST can be used as a promising food ingredient and protein source in the food system.

Keywords: Functional Properties, Fish Protein Hydrolysis, Response Surface Methodology, Skipjack Tuna By-products

## Introduction

Fish or other fishery products are important food ingredients for the community. Based on Food and Agriculture Organization (Marcio Castro de Souza, 2020) statistical data, more than 70% of caught fish are processed into canned fish, smoked fish, fillets, salted fish, frozen fish, and other curing products. Solid and by-products constitute 30–70% of whole fish, necessitating proper handling or utilization so that they do not cause any serious problems to the environment. Efforts to solve environmental problems associated with fish protein extraction not only help mitigate environmental and sustainability challenges but also increase the value of fish by-products and the efficiency of the fish industry (Khan *et al*., 2022; Korkmaz & Tokur, 2022; Marcio Castro de Souza, 2020). Valorization of fish by-products into highly valuable commodities has become an serious concern for researchers (Rodrigues *et al*., 2021; Nawaz *et al*., 2020; Riyadi *et al*., 2020a; Riyadi *et al*., 2020b). One way to valorize fish by-products is to process fish protein hydrolysates (FPH) from fishery by-products into functional food ingredients. The chemical composition, nutrient bioavailability, and bioactive compounds contained in FPH make FPH an important ingredient for the fortification of foods. Previous studies reported that fishery by-products (heads, skin, fins, bones, viscera, frames, trimmings, and scales) contain 8–35% crude protein, 0–25% fat, and 50–80% moisture (Ghaly *et al*., 2013; Sila & Bougatef, 2016; Saranya *et al*., 2018). The functional properties of FPH are important parameters for food systems, particularly when FPH is used as an ingredient in food. Among these important functional properties are protein solubility and viscosity, parameters that are related to the sensory characteristics of food colloid systems. Protein hydrolysates have been reported to have high

protein solubility, suggesting potential applications in the food industry (Nguyen Thi My Huong & Clair Donnay-Moreno, 2024; Amiza *et al*., 2019).

FPH can be produced from fishery by-products, such as the frames and trimmings of fish (Lee *et al*., 2011; Hou *et al*., 2011). One of the commercial fish species in Indonesia from which FPH can be produced is the skipjack tuna (*Katsuwonus pelamis*, Linnaeus 1758) (Yusuf *et al*., 2018). This species contains 24.13–25.29% crude protein, 0.41–0.60% fat, 1.43–1.49% ash, and 71.76–73.28% moisture (Nurjanah *et al*., 2015). A common method for producing FPH that is safe, easy to control, specific in breaking down peptides, non-toxic, and relatively cheap is enzymatic hydrolysis (Tavano, 2013). Factors such as pH, temperature, and time are important in a protein hydrolysis process (Gao *et al*., 2021; Korkmaz & Tokur, 2022). In addition, the selection of protease enzymes influences the FPH processing. In this research, papain was chosen for the production of FPH from skipjack tuna frames and trimmings due to its ready availability on the market, low price, and the lower relative bitterness resulted in the end product (Petrova *et al*., 2018).

The optimization conditions for FPH production from skipjack tuna by-products, such as frames and trimmings, using papain enzymes have yet to be established. Efforts to optimize variables such as pH, temperature, and time of hydrolysis are required to provide maximum response simultaneously. The optimization of PHST production can be approached using Response Surface Methodology (RSM), a collection of mathematical and statistical techniques based on matching polynomial equations with experimental data (Bezerra *et al*., 2008). RSM, a set of statistical methods for experimental design, response modeling, and level factor optimization (Raymond H.M & Montgomery, 2016), has been extensively used in experimental research, including in the food science of animal resources (Rheem, 2023). This study aimed to establish optimum conditions for producing protein hydrolysis of PHST with papain according to RSM. The resulted PHST was then analyzed for its characteristics, including degree of hydrolysis (DH), protein solubility, and viscosity. This research is expected to provide basic data for further research into chemicals, bioactive compounds, and their role in health.

## Materials and Methods

### Materials

The raw materials used in this study were frames and trimmings of skipjack tuna collected from the Fish Processing Center in Adisara, Jatilawang, Banyumas Regency, Central Java. These raw materials were stored in a styrofoam box, with the temperature maintained at 1–5°C by adding ice gel, for 90 minutes of transportation until reaching the laboratory. Upon reaching the laboratory, the raw materials were stored in a freezer (-18°C) before being processed into protein hydrolysates (PHST). Papain with an activity unit of 0.0835 ± 0.0009 U/mL was used for processing PHST. The materials used for analysis were 10% trichloroacetic acid (TCA), 0.1 M HCl (Merck), and 0.1 M NaOH (Merck). The analytical equipment used included water baths (Biobase-SY-2L4H), Kjeldahl equipment, and a viscometer (NDJ-5S series).

### The production of protein hydrolysates from skipjack tuna by-products (PHST)

PHST was produced according to Prasetyo et al., (2021) procedure with slight modifications. The raw materials were weighed at 50 --g, added with 150 mL of distilled water (1:3), and homogenized. The homogenate was added with papain at 5%. Samples were then grouped according to treatment: they were heated in a water bath (Biobase-SY-2L4H) at pH 6 ($A_1$) and 7 ($A_2$) and temperatures of 50°C ($B_1$), 60°C ($B_2$), and 70°C ($B_3$) for 90 minutes ($C_1$), 180 minutes ($C_2$), and 270 minutes ($C_3$). During hydrolysis, the pH was maintained at 6 and 7; acetic acid ($CH_3COOH$ - EMSURE) was added to make it acidic, and sodium hydroxide (NaOH 0.1M - MSURE) was added to make it basic. After heating, the enzyme was inactivated at 80°C for 30 minutes. The filtrate was filtered using filter paper (Whatman No. 40) for further analysis in the laboratory.

### Optimizing the hydrolysis conditions using Response Surface Methodology (RSM)

### Formulation and response design stage

Analysis of the influence of processing conditions, including pH, temperature, and time of hydrolysis, on PHST processing, specifically in terms of DH, protein solubility, and viscosity, was conducted using Response Surface Methodology (RSM) with the Design-Expert (DX) 13® software (Stat-Ease Inc., Minneapolis). Box-Behken Design (BBD) was the RSM design used in this research for randomization to determine the independent and fixed variables. The fixed variables in this study were degree of hydrolysis (DH), protein solubility, and viscosity, while the independent variables were pH ($A_1$ = 6 and $A_2$ = 7), temperature ($B_1$ = 50°C, $B_2$ = 60°C, and $B_3$ = 70°C), and time of hydrolysis ($C_1$ = 90 minutes, $C_2$ = 180 minutes, and $C_3$ = 270 minutes). Minimum and maximum limits were determined by trial

and error (Table 1). Fixed variables, along with minimum and maximum limit values, were entered into the DX software to be randomized, resulting in 18 treatment combinations for analysis (Table 2), with degree of hydrolysis, protein solubility, and viscosity being the measured and optimized responses.

### Response analysis stage

Each response variable was analyzed using ANOVA, with the ANOVA model being selected in accordance with what the program suggested. The ANOVA model selection was based on the highest significance value. The model that demonstrated significance in ANOVA and non-significance in the lack of fit test was selected to analyze the variables. The software analysis produced a normal plot of residuals, illustrating that the residuals (the difference between the actual response and the predicted response value) followed a normal line (straight line). Data points being closer to the normal line indicates that the data were spread normally. In other words, the actual results were close to the results predicted by the software (Nurmiah *et al*., 2013).

### Optimization stage

This stage aimed to optimize each response, resulting in a recommendation of several new optimal formulas according to the software. The most optimal formula was the one with the maximum desirability value. Desirability value is the value of the optimization objective function that shows the ability of the program to fulfill desires based on the criteria set for the final product. Ranging from 0 to 1.0, the value indicates the ability of the program to produce the desired product better (Riyadi *et al*., 2019)

Table 1. The ranges of independent variables

| Component | Independent Variable | Minimum | Maximum |
|---|---|---|---|
| A | pH | 6 | 7 |
| B | Temperature (°C) | 50 | 70 |
| C | Time of hydrolysis (minutes) | 90 | 270 |

### Degree of Hydrolysis (DH) Analysis

Twenty milligrams of sample was added to 20 mL of 10% trichloroacetic acid (TCA), mixed for 30 minutes, and centrifuged at 7.800 x g for 15 minutes. The supernatant formed was analyzed for its nitrogen content using the Kjeldhal method. The degree of hydrolysis was calculated based on (Hoyle & Merritt, 1994).

### Protein Solubility Analysis

The protein solubility of PHST was evaluated according to Islam *et al*. (2012) with slight modifications. One gram of PHST was dissolved in 100 mL of deionized water. The solution was adjusted to different pH levels (2, 4, 7, and 10) with either 0.1 M HCl or 0.1 M NaOH. It was then kept for 30 min at 30°C in a water bath (Biobase-SY-2L4H). The protein content in the supernatant was determined using the Kjeldhal method. Protein solubility is described as the amount of soluble protein from the total protein, calculated using the formula:

$$A = \frac{\text{Protein content in the supernatant}}{\text{Total protein content in the sample}} \times 100$$

### Viscosity Analysis

The viscosity of PHST was determined manually (viscometer, NDJ-5S series) and is described in percentages.

### Statistical Analysis

The equation model recommended by the software is based on the lack of fit value. The selected model was used for carrying out ANOVA. The model with a significant value for ANOVA and a non-significant value for lack of fit was selected to analyze the variables. The model was considered significant if the p-value was < 0.05, which was also reflected in the lack of fit. The accuracy level of the model equation recommended by the software was described by $R^2$ (coefficient of determination) and adjusted-$R^2$ values. After obtaining a recommended model, the next optimization step was to determine criteria, including variables and each influencing response. This stage also determined the goals, goal limits, and levels of importance. In the last stage, several optimization solutions with different desirability levels were presented. The optimal solution had a desirability value close to 1 and was selected as the best condition for optimizing the production of PHST.

## Results and Discussion

### Optimization Conditions Based on RSM

The results of laboratory analysis of DH, protein solubility, and viscosity are shown in Table 2. These results were inputted to the BBD RSM software, and the analysis results are shown in Table 3. A total of 16 experiments with various independent variables were selected as optimization dependent variables.

Optimization using RSM has several benefits, including less experiment runs needed, less process parameters, less amount of raw material, and less space and operator requirements (Daud *et al*., 2021).

ANOVA and regression analysis were carried out, and the results are presented in Table 4. The significance of each independent variable was evaluated using its p-value. All p-values were lower than 0.05, suggesting that all quadratics were significant. Lack of fit described the model's ability to measure errors that existed due to deficiencies in the model. In the lack of fit value doesn't appear (Table 4), indicating that the model was neither significant nor adequate to explain the data. Meanwhile, errors illustrate the diversity of data (Faisal *et al*., 2023).

Table 2. DH, protein solubility, and viscosity of PHST

| Formulation | Factors | | | Responses | | |
|---|---|---|---|---|---|---|
| | pH | Temperature (°C) | Time of Hydrolysis (minutes) | Degree of Hydrolysis/ DH (%) | Protein Solubility (%) | Viscosity (cP) |
| 1 | 6 | 50 | 90 | 45.657 ± 3.416 | 37.365 ± 0.926 | 3.7 ± 0.14 |
| 2 | 6 | 50 | 180 | 61.702 ± 2.419 | 39.61 ± 0.735 | 3.1 ± 0.14 |
| 3 | 6 | 50 | 270 | 74.254 ± 2.908 | 40.13 ± 0.438 | 3.05 ± 0.07 |
| 4 | 6 | 60 | 90 | 51.210 ± 3.117 | 39.465 ± 0.912 | 4.1 ± 0.28 |
| 5 | 6 | 60 | 180 | 83.813 ± 0.989 | 50.375 ± 0.092 | 3.7 ± 0.14 |
| 6 | 6 | 60 | 270 | 85.837 ± 0.235 | 51.785 ± 0.884 | 3.55 ± 0.21 |
| 7 | 6 | 70 | 90 | 67.985 ± 0.146 | 48.865 ± 0.318 | 4.15 ± 0.07 |
| 8 | 6 | 70 | 180 | 78.280 ± 1.063 | 50.685 ± 0.841 | 4 ± 0.14 |
| 9 | 6 | 70 | 270 | 63.874 ± 2.326 | 47.07 ± 0.764 | 3.5 ± 0.28 |
| 10 | 7 | 50 | 90 | 64.785 ± 1.885 | 41.805 ± 2.383 | 3.25 ± 0.07 |
| 11 | 7 | 50 | 180 | 75.518 ± 1.684 | 45.025 ± 0.898 | 3.05 ± 0.07 |
| 12 | 7 | 50 | 270 | 78.969 ± 0.095 | 49.36 ± 0.891 | 2.95 ± 0.07 |
| 13 | 7 | 60 | 90 | 80.670 ± 2.349 | 38.98 ± 0.156 | 5.36 ± 0.06 |
| 14 | 7 | 60 | 180 | 86.973 ± 1.912 | 49.095 ± 0.092 | 4.85 ± 0.07 |
| 15 | 7 | 60 | 270 | 87.667 ± 2.344 | 46.505 ± 0.361 | 3.75 ± 0.07 |
| 16 | 7 | 70 | 90 | 71.153 ± 3.201 | 36.435 ± 1.959 | 4.75 ± 0.07 |
| 17 | 7 | 70 | 180 | 81.068 ± 1.498 | 38.6 ± 1.188 | 3.85 ± 0.07 |
| 18 | 7 | 70 | 270 | 69.110 ± 1.266 | 35.98 ± 0.608 | 3.65 ± 0.35 |

Table 3. Running BBD RSM for PHST

| Run | Factors | | | Responses | | |
|---|---|---|---|---|---|---|
| | pH | Temperature (°C) | Time of Hydrolysis (minutes) | Degree of Hydrolysis/DH (%) | Protein Solubility (%) | Viscosity (cP) |
| 1 | 6 | 60 | 270 | 85.837 | 51.785 | 3.5 |
| 2 | 7 | 70 | 180 | 81.068 | 38.6 | 3.8 |
| 3 | 6.5 | 70 | 270 | 68.874 | 47.07 | 3.5 |
| 4 | 6.5 | 60 | 180 | 83.813 | 50.375 | 3.7 |
| 5 | 6.5 | 60 | 180 | 83.813 | 50.375 | 3.7 |
| 6 | 6.5 | 50 | 270 | 74.254 | 40.13 | 3.05 |
| 7 | 7 | 60 | 270 | 87.667 | 46.505 | 3.75 |
| 8 | 6 | 50 | 180 | 61.702 | 39.61 | 3.1 |
| 9 | 6 | 70 | 180 | 78.28 | 50.685 | 4 |
| 10 | 6.5 | 60 | 180 | 83.813 | 50.375 | 3.7 |
| 11 | 6.5 | 70 | 90 | 67.985 | 48.865 | 4.15 |
| 12 | 7 | 60 | 90 | 80.67 | 38.98 | 5.36 |
| 13 | 6 | 60 | 90 | 51.21 | 39.465 | 4.1 |
| 14 | 7 | 50 | 180 | 75.518 | 45.025 | 3.05 |
| 15 | 6.5 | 60 | 180 | 83.813 | 50.375 | 3.7 |
| 16 | 6.5 | 60 | 180 | 83.813 | 50.375 | 3.7 |

Table 4. Model analysis for DH, protein solubility, and viscosity

| Responses | Model | Significant ($p < 0.05$) | Lack of fit ($p < 0.05$) | $R^2$ | Adjusted $R^2$ | CV (%) | Adeq Precision |
|---|---|---|---|---|---|---|---|
| Degree of Hydrolysis | Quadratic | < 0.0001 | - | 0.9800 | 0.9543 | 3.51 | 21.0828 |
| Protein Solubility | Quadratic | 0.0317 | - | 0.8500 | 0.6572 | 6.85 | 6.4925 |
| Viscosity | Quadratic | 0.0181 | - | 0.8748 | 0.7138 | 7.57 | 10.0574 |

The model equation suggested by ANOVA was:

$$DH = 83.81 + 5.99A + 4.88B + 8.89C - 2.76AB - 6.91AC - 6.93BC + 1.24A^2 - 10.91B^2 - 8.71C^2 \quad 1)$$

$$\text{Protein solubility} = 50.38 - 1.55A + 2.89B + 2.60C - 4.38AB - 1.20AC - 1.14BC - 3.03A^2 - 3.86B^2 - 3.16C^2 \quad 2)$$

$$\text{Viscosity} = 3.70 + 0.1575A + 0.3188B - 0.4388C - 0.0375AB - 0.2525AC + 0.0000BC + 0.1825A^2 - 0.3950B^2 - 0.2950C^2 \quad 3)$$

where A = pH, B = temperature, and C = time of hydrolysis.

The $R^2$ value for DH showed that 98% of the total variance was explained by the model. Meanwhile, for protein solubility and viscosity, the model could explain 85% and 87% of the total variance, respectively. The higher the $R^2$ value, the higher the correlation between the experiment and the predicted value of the response variable (Said & Sarbon, 2020; Ekpenyong *et al*., 2017). These figures showed that the response variables could be used to predict PHST production. The adjusted $R^2$ value calculated was close to 1, indicating the suitability of the model for predicting experimental data (Ma *et al*., 2009). A low coefficient of variation (CV) found in this study indicated that the repeatability of the experiment data was very good. Adeq. precision is the signal-to-noise ratio, which ideally should exceed 4. According to Table 4, the adeq. precision was greater than 4 for all responses. The coefficient of determination ($R^2$) and non-significant lack of fit value, as shown in Table 4, demonstrated the significance of the model and the fitness of experimental values to the theoretical values predicted by the model's regression equation, respectively.

The formulated conditions of the PHST optimization process are shown in Table 5. A desirability value of 0.906, which was close to 1 (100%), showed a high level of desirability. This value served as an indicator for setting limits, allowing for the determination of the best value for each response variable in the optimization process (Luis Pérez, 2021). It indicated the closeness of the response to the target (Winarni *et al*., 2021). RSM use in optimization could explain the variance in variables that could influence the overall response design (Choudhary & Pramanik, 2021).

### Degree of Hydrolysis

Degree of hydrolysis (DH) is described as the ratio of the number of peptide bonds that have been successfully broken to the total number of peptide bonds in the original protein. Based on Table 3, the higher the DH, the higher the temperature and time of hydrolysis. In this study, the DH of PHST ranged from 51.21% to 87.667%. For comparisons, the DHs of protein hydrolysates from tuna heads treated with proteases from *Bacillus mojavensis* A21 and Alcalase at 100 minutes were 13% and 9%, respectively (Bougatef et al., 2012). Taheri & Bakhshizadeh G (2020), also reported that the hydrolytic activity of pepsin increased in the initial 50 minutes of incubation before reaching a slower phase, followed by a stable phase at 150 minutes. Peptide cleavage occurred in the initial 50 minutes of the reaction with a DH of 10% at 60 minutes, which increased to 21.7% at 150 minutes before entering a steady phase.

Based on 3D plots for DH (Figure 1a), in the initial stage (temperatures of 50–60°C for 90–180 minutes), DH climbed until it approached the optimum point (flag). From that point on, DH activity decreased. This finding is similar to (Auwal et al., 2017) report that the DH of stone fish protein hydrolysates increased with time of hydrolysis in bromelain. The DHs at 50 to 100 minutes ranged between 20% and 22% (Fraterrigo Garofalo *et al*., 2023). Similar results were reported by (Saidi *et al*., 2016), who showed that there was a significant increase during the first hour of tuna-byproduct hydrolysis, and no significant variations could be observed thereafter. Temperature has an influence on the reaction rate during protein hydrolysis, the activation energy of the catalytic reaction, and the thermal stability of enzymes and substrates (Shen *et al*., 2012). Several researchers, including Ovissipour *et al*. (2012); Prabha *et al*. (2013); Klomklao & Benjakul (2017); and Korkmaz & Tokur, (2022); have reported that DH increased with increasing temperature and time of hydrolysis until it reached the optimal limit. Above the optimal temperature, DH decreased because higher temperatures caused protein denaturation.

DH is also influenced by pH, which influences enzyme activity by modulating the hydrophobic and hydrophilic interactions of peptides (Finkler et al., 2022). As reported by Shen *et al*., (2012) low and high pH conditions cause a decrease in enzyme activity because the active site becomes increasingly distorted, which has a bad effect on enzyme function. The papain enzyme is active at pH 6.5–7.0 (Aluko, 2017). In this research, the pH used was 6–7. Enzymes have catalytic active sites with charged amino acids, whose dissociation state can be influenced by pH by changing the iconic bonds that maintain the three dimensional shape of proteins. This effect causes changes in protein function or enzyme interaction (Shu *et al*., 2016).

Table 5. Formulation of the optimization process based on BBD RSM

| pH | Temperature | Time of Hydrolysis | Degree of Hydrolysis | Protein Solubility | Viscosity | Desirability |
|---|---|---|---|---|---|---|
| 6.386 | 61.190 | 228.540 | 85.680 | 51.538 | 3.587 | 0.906 |

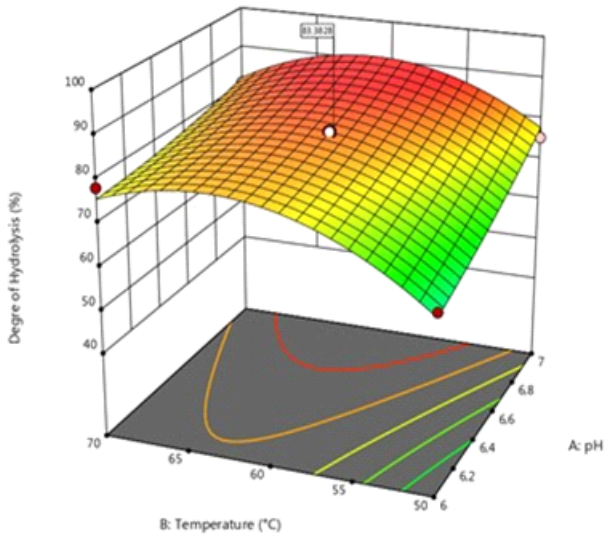


Figure 1a. Response surface graph for the degree of hydrolysis as a function pH, Temperature and Time of hydrolysis from PHST.

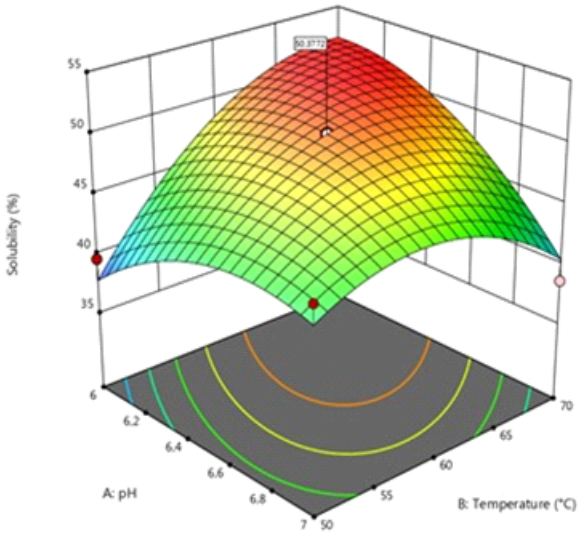


Figure 1b. Response surface graph for the protein solubility as a function pH, Temperature and Time of hydrolysis from PHST.

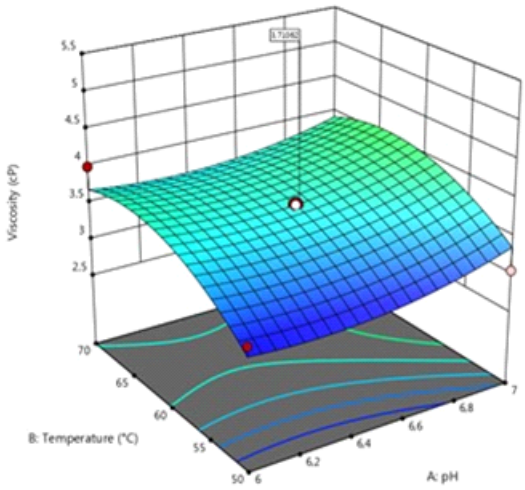


Figure 1c. Response surface graph for the viscosity as a function pH, Temperature and Time of hydrolysis from PHST.

Therefore, to obtain optimal conditions, including temperature, pH, and hydrolysis time, which are important to obtain the optimal DH, protein solubility, and viscosity for PHST production (Figure 1a), experiments with RSM were carried out. The optimum conditions based on RSM are presented in Table 5. Valencia *et al.* (2014), also reported the mechanism responsible for the shape of the curve of fish protein hydrolysis with Alcalase, which is characterized by an initial "hydrolysis phase" followed by a slowdown and stabilization of DH.

### Protein Solubility

Protein solubility is considered by many researchers to have the most important functional properties because it significantly affects all others (Benjakul *et al.*, 2014). It is directly correlated with degree of hydrolysis, where the greater the DH the greater the protein solubility. Prasetyo *et al.* (2021) reported that the protein solubility of protein hydrolysates from tilapia frames with 87–89% DH was 65.22–67.83%. Latorres *et al.* (2018) described that the solubility of protein

stage, protein solubility increases until it reaches the optimum point. Beyond that, it decreases.

Protein solubility is also affected by pH conditions during hydrolysis; solubility is higher at pH above 8, while at pH 5 solubility is very low. The lowest precipitation and solubility of fish protein occur at the isoelectric point in the pH range of 5.3–5.5 (Ben Khaled *et al*., 2014; Alinejad *et al*., 2017). In some cases Chalamaiah *et al*. (2015), the protein solubility at pH 2, 3, 4, and 5 yields lower results when compared to that at pH 8, 9, 19, 11, and 12. According to Bougatef *et al*. (2012), a very high level of hydrolysis is not good because it will have a negative effect on the functional properties, so that the substrate not to be hydrolyzed extensively. It is necessary to keep the level of hydrolysis used at the right level.

### Viscosity

Based on several 3D plots (Figure 1c), a quadratic model with a highly explained variance for viscosity was established ($R^2$=0.8748, p<0.005). The values of viscosity ranged from 3.05 to 5.36cP. Table 2 shows that temperature, hydrolysis time, and DH were negatively correlated with viscosity. The 3D plots of viscosity (Figure 3c) show that viscosity increased in early minutes. However, it decreased with increasing hydrolysis time. This was due to the fact that, as temperature and hydrolysis time increase, the protein molecules and their weights in the solution become less uniformly distributed. According to Chiodza & Goosen (2023), viscosity is expected to continue to decrease as the molecular weight of the peptide decreases with enzyme activity. Viscosity can decrease with higher temperature. Ahmad *et al*. (2019), reported findings that temperature reduces viscosity in the rubbery phase.

Hydrolysis breaks protein bonds with a large molecular weight into constituent components with a lower molecular weight. The longer the hydrolysis process takes place, the more peptide molecules with a smaller molecular weight are produced; this affects the hydrogen bonds of the peptide with water (Castro & Sato, 2014). Annisa *et al*. (2017) found that the viscosity of hydrolysates from tilapia (*Oreochromis niloticus*), milkfish (*Chanos chanos*, Froskal 1755), and shark (*Hemigaleus balfouri*) derived through hydrolysis at 55°C using papain enzyme (5%) for six hours were 1.91cP, 1.81cP, and 1.31cP, respectively. This shows that the longer the hydrolysis process, the lower the viscosity value. In addition, Prasetyo *et al*. (2021) reported that the viscosity of protein hydrolysates from tilapia frames ranged from 2.35 to 3.60 cP.

## Conclusion

At pH of 6.386 and a temperature of 61.190°C, and with a hydrolysis time of 228.540 minutes, the optimization of protein hydrolysis using skipjack tuna by-products with 5% papain enzyme can yield a desirability value of 0.906. These conditions are recommended for further research into food fortification and functional food development.

## Acknowledgments

We would like to thank the Ministry of Finance and the Ministry of Education, Culture, Research, and Technology of the Republic of Indonesia (LPDP – BPI, with contract number 0773/J5.2.3/BPI.06/10/2021) for funding this Doctoral Research.

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