

Original Paper

# The Optimization of Catfish Smart Flavor Production by Biduri and Papain Enzymatic Hydrolysis

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**Abstract**—The consumption of Monosodium Glutamate with a large amount can lead to nerve cell damage to the brain so that natural ingredients substitute MSG is needed. In this research, we produced smart flavors from catfish through enzymatic hydrolysis by combining papain and biduri enzymes. The purpose of the study was to identify the influence of enzyme concentration and length of hydrolysis on the smart flavor characteristics and determine the best treatment to produce smart flavors. The parameters identified were color, yield, moisture content, dissolved proteins, degrees of hydrolysis, antioxidants, water binding ability, and emulsion stability. The results show the highest brightness are biduri and papain combination by 60:40 with one-hour hydrolysis. The highest dissolved protein is 50:50 combination with three-hour hydrolysis. In addition, antioxidant activity is marked in a combination of 50:50 with one-hour hydrolysis.

**Keyword:** Biduri, hydrolysis, smart flavor, papain

## I. INTRODUCTION

Indonesian people have an excessive interest in the taste of umami food. Umami flavors are produced by MSG (Monosodium Glutamate) which is a synthetic flavoring ingredient. The safe limit of consumption of Monosodium Glutamate according to [1] is 16 mg/kg of body weight per day. According to [2] quoted [3], the consumption of Monosodium Glutamate in large quantities can cause nerve cell damage in the brain. Damage to nerve cells in the brain of mice is identified when given MSG at a high enough dose (0.5 g / kg of body weight per day), so it is necessary to develop natural ingredients substitute MSG known as smart flavor.

Smart flavors are produced with the main ingredient of catfish through the process of hydrolysis. The process of hydrolysis involves protease enzymes namely papain and biduri. Biduri proteases are exopeptidase enzymes (broken down at the end of protein chains) and papain enzyme endopeptidase (broken down in the middle of the protein chain). Previous research results reported a combination of

biduri and papain enzymes as much as 0.2% with a ratio of 3: 3 for 3 hours, which has been found to produce the best sword flavor with dissolved protein levels of 0.157, color (brightness level) of 0.009 and fondness value of 0.088, so that the total effectiveness value is highest [4]. Enzyme concentration and hydrolysis time affect the characteristics of hydrolysate produced and are thought to affect the characteristics of smart flavors. The purpose of the study is to know the influence of enzyme concentration and length of hydrolysis on the characteristics of smart flavors and know the best treatment to produce high-quality smart flavors.

## II. MATERIAL AND METHOD

Research materials are catfish from Tanjung Jember Market, coarse extract of biduri enzyme from Watu Ulo Beach in Jember regency, coarse extract of papain enzymes, akuades, sugar, salt, garlic powder, STTP, and CMC. The chemical used buffers phosphate 0.05 pH 7, aquades, Lowry reagents of hexant solvents, K<sub>2</sub>SO<sub>4</sub>, CuSO<sub>4</sub>, selenium, H<sub>2</sub>SO<sub>4</sub> concentrated, NaOH 40%, H<sub>3</sub>BO<sub>3</sub>, bromine green cresol in pink, HCl 0.02 N, tyrosine 0.1 Mm, TCA (trichloroacetic acid) 0.1 M, casein, Na<sub>2</sub>CO<sub>3</sub> 0.4 M, reagent DPPH, Follin ciocalteau, and ethanol p.a. ethanol 70%.

### A. Production of biduri and papain enzymes

The resulting sap was added with a phosphate buffer of 0.05 M pH 7 with a ratio of 1:1. The next process was centrifuge with a temperature of 4°C at 8000 rpm for 10 minutes in order to produce supernatant.

### B. Production of hydrolysate catfish protein

Catfish was prepared in fillets to separate the meat from its head, bones, tail, and contents. The catfish meat weighed 50 grams and was crushed using a stainless steel blender with the addition of a 1:2 aquades (b/v). After that, a mixture of 2 ml biduri and papain enzymes was mixed evenly. The comparisons of biduri enzymes and papain enzymes used ranged from 40:60, 50:50, to 60:40. The hydrolysis process

was performed in water bath at a temperature of 55°C for 1 hour, 2 hours, and 3 hours. The mixture was warmed at a temperature of 90°C for 20 minutes aimed at inactivating protease enzymes. The next sample was concentrated at 4°C at 3500 rpm for 15 minutes.

#### C. Production of smart flavor made of hydrolysate catfish protein

The manufacture of smart flavor from catfish hydrolysate was done by adding 50 grams hydrolysate of catfish, which was combined with 25% sugar, 25% salt, 5% garlic powder, 5% STTP, and 40% CMC of the weight of fish. The sample was dried at a temperature of 60°C for 24 hours. The samples that had been dried were blended and soaked with a syring of 80 mesh.

#### D. The characteristics of smart flavors

##### a. Yield

Yield is the percentage of products obtained from the final weight. The yield was determined by calculating/weighing the final weight of the product resulting from the process. The formula for calculating the yield was as follows:

$$\text{Yield (\%)} = \frac{\text{weight after processing(g)}}{\text{initial weight (g)}} \times 100\% \quad (1)$$

##### b. Color

Color testing was conducted by the Fardiaz method [5] using a color reader. The dL value which was identified was then recorded [17]. The value L\* (Lightness) indicates the brightness level with a range of 0 = dark to 100 = light. The value of L\* can be obtained by the following calculations:

$$L^* = \frac{94,35}{L_{\text{standard}}} \times L_{\text{sample}} \quad (2)$$

##### c. Water content

Water content was tested using the Sudarmadji method [6]. Water content testing began by weighing an empty cup that had been put in the oven for 2 hours and placed in a decibel as a (gram). Then 2 grams of the sample was put in a cup and weighed as b (gram). The cup containing the sample was put into the oven for 24 hours, and it was cooled in a desiccator and weighed. This treatment was repeated until it reached a constant weight as c (gram). The formula for calculating water content is as follows:

$$\text{Water content (\%)} = \frac{b-c}{b-a} \times 100\% \quad (3)$$

##### d. Protein Content

Lowry's dissolved protein began with a sample weighing 0.1 g and dissolved with a 10 ml aquades. The sample was centrifuged for 5 minutes, and 0.125 ml of filtrate was taken and reacted with a 2.5 ml Mix-Lowry reagent. Subsequently, it was kept for 10 minutes. Next, it involved adding 0.25 ml Follin and let stand for 30 minutes. Add a aquades to a volume of 5 ml and measure the absorbance with a wavelength of 750 nm.

##### e. Degree of hydrolysis

The analysis of free amino groups was used in determining the magnitude of hydrolysis (DH) using the modified Adler-

Nissen TNBS method [7] [16]. These samples involved 0.25 ml and 2 ml buffer phosphate pH 8.2 and 2 ml solution Trinitrobenzene sulfonic acid (TNBS) 0.01% (v/v). Homogenization was performed for 1 hour at a temperature of 50°C in a closed water bath. 4 ml HCl solution 0.1 N was added afterward, and then the sample was cooled and incubated for 30 minutes at room temperature in a dark room. Absorbance measurement was conducted at a wavelength of 340 nm. The calculation of hydrolysis degrees is based on the following formula:

$$DH = \frac{AN_2 - AN_1}{N_{pb}} \times 100\% \quad (4)$$

##### f. Power and stability of the emulsion

Measurement of the power and stability of the emulsion was carried out using the Parkington et al. method [8]. A sample of 0.1 grams was tethered to a 100 ml buffer of phosphate 0.05 M pH 7. The solution was stirred using a stirrer for 15 minutes. At this point, 25 ml cooking oil was put into the solution, and it was stirred with a blender for 3 minutes. The measurement of emulsion power was done by taking 1 ml of blended suspension, while emulsion stability measurement was done by taking 1 ml of suspension is kept for 10 minutes. Each suspension is added by 5 ml SDS 0.1% and homogenized and absorbance measuring with a wavelength of 500 nm. The emulsion power formula is:

$$EAI (m^2/g) = \frac{2 \times 2,303}{cx(1-\emptyset) \times 10} \times \text{abs} \times \text{dilution} \quad (5)$$

While the emulsion stability formula is:

$$ESI (\text{minute}) = \frac{T \times \Delta t}{\Delta T} \quad (6)$$

##### g. The Determination of antioxidant activity

Antioxidant activity was measured by employing [9]. Dilution of 0.00195 g DPPH was performed into 50 ml of ethanol p.a. The sample was diluted by putting 0.01 g of powder into 10 ml of 70 ml ethanol. A total of 1.5 ml of sample solution was tethered to 1.5 ml of DPPH. The solution was secretive and was let stand for 30 minutes at 37°C in the dark. The solution was measured to identify absorbance by using a UV VIS spectrophotometer with a wavelength of 515 nm. How to calculate antioxidant activity is actualized by referring to the formula below:

$$DPPH \% = \frac{A_{\text{blanko}} - A_{\text{sampel}}}{A_{\text{blanko}}} \times 100 \quad (7)$$

#### E. Data analysis

The data of the study was calculated to identify the average and the standard deviation. The results obtained were then presented in diagrams or tables. All data processing results presented in the form of diagrams or tables were analyzed descriptively.

### III. RESULT AND DISCUSSION

#### A. Color

Color tests were performed on brightness (lightness) using a color reader. The analysis results showed that the highest L levels were indicated by smart flavor samples with a

comparative treatment of the concentration of the enzyme biduri:papain (60:40) with one-hour hydrolysis. Meanwhile, the lowest L level was shown by the sample with a comparative treatment of the concentration of the enzyme biduri: papain (40:60) with 3-hour hydrolysis.

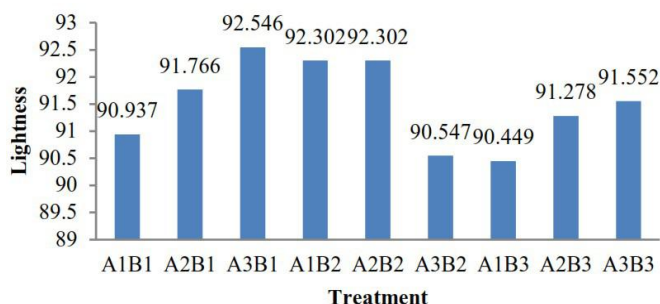


Fig. 1. Lightness data of catfish smart flavor. Biduri and papain enzyme concentration ratio (A1=40:60, A2=50:50, A3=60:40) and hydrolysis time (B1= 1 hour, B2= 2 hours, B3= 3 hours).

The color formed in smart flavors resulted from the hydrolysis where the peptide bond was broken by protease enzymes producing amine groups which was a prequel to the Maillard reaction. In this condition, the protein of the mine group would react with the aldehyde or ketone group of reducing sugars and will produce a brown color. The longer the hydrolysis time, the result of the smart flavor product produced the darker (browning) was identified. This happened because the longer the hydrolysis time, the more Maillard products so that the color of the catfish smart flavor was getting darker [10]. Meanwhile, the concentration comparison between the enzyme of biduri and papain used had no effect on the brightness of the smart flavor produced [4].

### B. Protein Content

Smart flavor possessed dissolved protein, which was the content of proteins dissolved in smart flavors. The analysis results showed that the highest protein levels were obtained from the treatment of concentrations of enzyme biduri:papain (50:50) with a three-hour hydrolysis. The resultant protein level was 1,363 gr/ml, while the lowest dissolved protein levels were obtained from the treatment of the concentration of the enzyme biduri: papain (60:40) with a one-hour hydrolysis, which gained protein level of 0.93 gr/ml.

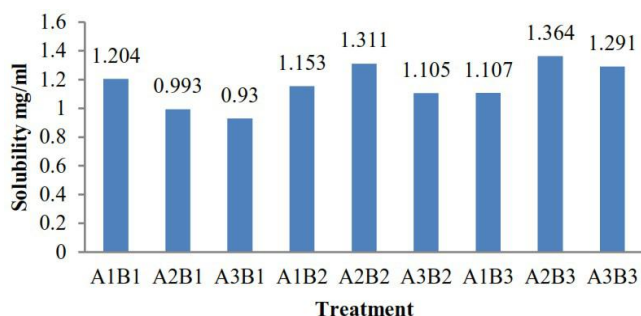


Fig. 2. The dissolved protein content of catfish smart flavor. Biduri and papain enzyme concentration ratio (A1=40:60, A2=50:50, A3=60:40) and hydrolysis time (B1= 1 hour, B2= 2 hours, B3= 3 hours).

Papain enzyme is an endopeptidase enzyme that cuts the peptide bond in the inside or middle of the peptide bond chain so that it can produce peptide bonds with shorter chains. Increased concentration of papain enzymes used will increase short-chain peptide bonds, causing the biduri enzyme to break the peptide bond on the outside faster. As a corollary, more peptide bonds are broken into simple peptides and increase dissolved proteins [4]. The analysis results, which showed that the higher the concentration of papain used in the hydrolysis process for 1 hour, which led to the levels of dissolved protein smart flavor catfish. Meanwhile, at the time of hydrolysis of 2 and 3 hours, the most optimal ratio of biduri and papain enzymes occurred at a concentration ratio of 50:50. This happens because the work of the biduri and papain enzymes has reached equilibrium so that if the concentration of papain is raised, it will cause inaccuracy. Previous research has also shown that the ratio of the enzyme concentration of biduri:papain of 50:50 also shows the value of the most inflammatory dissolved protein levels because in the comparison of concentrations the work of enzymes has reached equilibrium [4].

### C. Antioxidant activity

Antioxidants are compounds that can inhibit oxidation reactions or neutralize free radicals. Antioxidant activity was tested using DPPH radicals expressed in the percentage of radical inhibition [15]. The greater the percentage of radical inhibition, the higher the antioxidant activity in a material was identified. This was marked by decreased absorption of DDPH solution to samples tested on spectrophotometers with a wavelength of 515 nm. The analysis results showed that the highest antioxidant activity in catfish smart flavors was indicated by samples with the treatment of biduri:papain enzyme concentration (50:50) with a hydrolysis time of 1 hour.

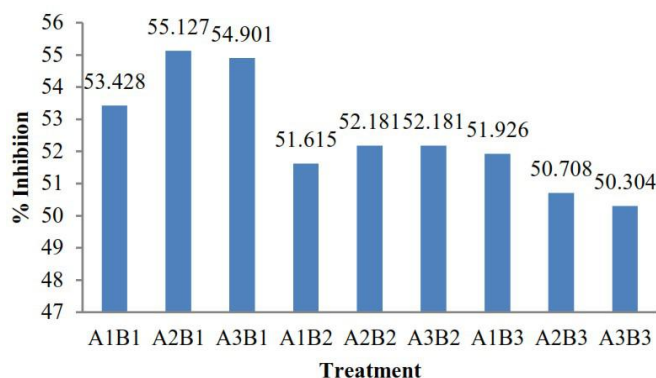


Fig. 3. Antioxidant activity of catfish smart flavor. Biduri and papain enzyme concentration ratio (A1=40:60, A2=50:50, A3=60:40) and hydrolysis time (B1= 1 hour, B2= 2 hours, B3= 3 hours).

The analysis results showed that hydrolysis experienced an increase in antioxidant activity in line with the increased concentration of papain used. However, at the time of hydrolysis 1 and 2 hours, there was a decrease in antioxidant activity in the ratio of the concentration of biduri: papain 40:60. This is also the case in a study conducted by [11] where

the antioxidant activity of catfish hydrolysate increased along with the increased concentration of the enzyme papain used, but to some extent, the addition of enzyme concentration decreased antioxidant activity.

Overall the results demonstrated that the antioxidant activity was unstable. This suggests that the difference in concentration and length of hydrolysis does not necessarily result in high antioxidant activity. According to [12] antioxidant activity is influenced by the molecular weight and composition of amino acids formed during the hydrolysis process. Hydrolyzed proteins produce amino acids, but there are some proteins that produce protein molecules [13].

#### D. Yield

The hydrolysis was carried out by using enzymes, which aided in converting the substrate into a hydrolysate product. The percentage of the number of products against the number of raw materials is called yield. The nutritional components dissolved in hydrolysates such as fats, proteins, and minerals during the hydrolysis process affect the magnitude of the yield of the products produced [14].

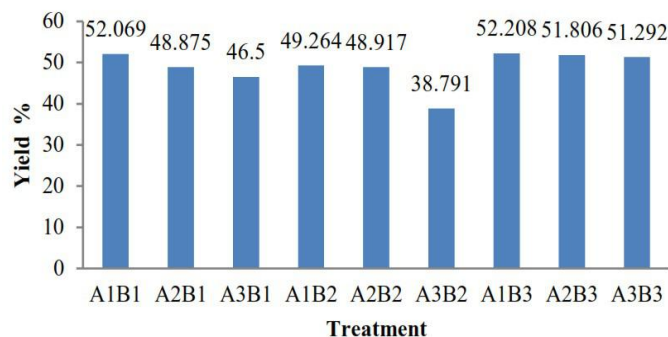


Fig. 4. The yield of catfish smart flavor. Biduri and papain enzyme concentration ratio (A1=40:60, A2=50:50, A3=60:40) and hydrolysis time (B1= 1 hour, B2= 2 hours, B3= 3 hours).

The highest yield is produced by smart flavor with a comparative treatment of biduri enzyme concentration: papain 40:60 with three-hour hydrolysis. Decreased yield value was marked along with the decreased concentration of papain enzyme used. This result was similar to the [14] claiming that a decrease in the yield value of milk fish fish is in line with the declining concentration of papain enzymes used. The timing of hydrolysis also affects the yield value of the resulting product. Smart flavors that are whitewashed for 3 hours tend to have a high yield value the hydrolysis process runs at its maximum compared to hydrolysis using 1 or 2 hours. A maximum hydrolyzed substrate will increase the yield value of the resulting product.

#### IV. CONCLUSION

The highest brightness is found in the mixture of biduri and papain at a ratio 60:40 with one-hour hydrolysis. The dissolved protein was 50:50 for 3 hours hydrolysis, while the highest antioxidant activity was marked at a ratio of 50:50 with one-hour hydrolysis.

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