Effect of enzyme papain against natural flavor of raw meat waste laundering surimi

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Abstract. Washing waste material surimi fish meat has the potential to be developed into a natural flavor because it contains of proteins and amino acids are quite high. Enzymatic hydrolysis of proteins can produce compounds savory (umami) which can be used as natural flavor. This study aimed to determine the effect of the enzyme papain as an agent of the natural flavor of the washing waste material surimi fish meat. The treatment includes control of 0% (without the enzyme papain), the addition of the enzyme papain 2%, 4% and 6%. The results showed that administration of different concentrations of the enzyme papain give effect to the yield and the value of the resulting organoleptic flavor. The yield of flavor respectively - helped by 6.18%, 7.23%, 9.98% and 12.03%. Best papain enzyme concentrations in this study were treated with the addition of P2 which papain enzyme 4%, note the value yield of 9.98% with an average rate of acceptance of the panelists of the color, aroma, and flavor are 6; 3.4 and 3.7 which means rather liked, a bit fishy, and rather tasteless fish, the protein content of 15.62%, 0.26% glutamic acid, and water solubility of 96.5%.

1. Introduction
Surimi product demand continues to increase, it is certainly going to continue to spur the interest of industrial enterprises in the production of surimi. According to Pangsorn et al [1] states that the production of surimi in the Southeast Asian region in 2005 is estimated at 315.800 tonnes. Washery waste material surimi fish meat (leaching) is known to contain lots of protein compounds. Park [2] mentions that washing waste content of fish meat such as sarcoplasmic proteins, lipids, and heme proteins. Trilaksani et al [3] reported that the wash water tilapia fish meat protein concentrate by 72.12% (w/w). According to Lioe and Yasuda [4] enzymatic hydrolysis of proteins can produce compounds savory (umami) which can be used as natural flavor. One enzyme which has protease activity, and has been widely used in the manufacture of enzymatically flavor is the enzyme papain. Therefore, the development of the utilization of waste leaching surimi as natural flavor materials enzymatically using the enzyme papain needs to be done in an effort to increase the value-added products made from waste.

2. Material and methods
2.1 Preparation of flavor
A sample of wastewater from the first stage fish meat washing process in the manufacture of kurisi fish surimi. Samples were frozen at -20°C before being taken to the laboratory. Preliminary research
conducted by measuring the pH value and the protein content of the waste. Making the flavor powder in the study include the concentration of materials is by heating the material at a temperature of 80°C until the volume of material to be half of its original volume. After going through the process of heating the sample, then hydrolyzed using the enzyme papain with different concentrations (0%, 2%, 4% and 6%) at a temperature of 55°C for two hours and continued with the process of inactivation of the enzyme papain at a temperature of 85°C for 15 minutes. Then dried the samples using an oven with a temperature of 60°C for 18 hours until a sample of flavor is found in a dry form.

2.2 Appearance test
Organoleptic testing is used to test hedonic scale which is the preference level of the color and quality of hedonic scale test for aroma and taste. This test uses untrained panelists as many as 30 people. In the organoleptic test of the flavor and aroma of the sample is dissolved in hot water, with a ratio of 1 gram sample of the flavor in 100 ml of water. The values given in the form of ranking starts from a very well-liked (score 9), until a very unwell-liked (score 1).

2.3 Protein test
Protein testing using [5]. The sample is weighed amount (1-2 g) and then put in a Kjeldahl flask, then added 1.9 g K2SO4, 40 mg HgO and 2.0 ± 0.1 ml of sulfuric acid concentrated and then boiled until a clear liquid. This clear solution was then transferred into a distillation apparatus, under the condenser placed 5 ml Erlenmeyer containing H3BO3 solution and 2-4 drops of indicator (a mixture of 2 parts of methyl red 0.2% and 1 part methylene blue 0.2% in alcohol). Condenser tube ends must be submerged in a solution of H3BO3. The solution in the flask was diluted to 50 ml and titrated with 0.02 N HCl until the color changes to gray. The same thing is done against a blank.

\[
P (%) = \frac{(V_A-V_B) \times N \times HCl \times 1000}{w \times 1000}
\]

P  = Levels of Protein
VA  = HCl to titrate sample (ml)
VB  = HCl to titrate blank (ml)
N  = Normality HCl (standard used).
w  = weight of sample (g)

2.4 Test Glutamic Acid Levels
Glutamic acid content measurement was conducted by [6]. A sample of 0.1-0.2 g inserted into the Kjeldahl flask and added 5 g of a mixture of salen and 20 ml of sulfuric acid technical concentrated then heated in the hood, the small flame with a shake. Fire was brought up for 5-10 minutes. The samples were heated to liquid color to green, and then cooled. Cold sample was diluted with 50 ml of water and transferred into a 250 ml boiling flask. The sample was then added 40 ml NaOH 40% and are connected by means of distiller for 50 minutes and then collected the distillate H3BO3%, and in titer with 0.1 N HCl then calculated using the formula:

\[
M = \text{Levels of glutamic acid}
N = \text{Content nitrogen}
V = \text{HCl (ML)}
N = \text{HCl 0, 1 N}
q = \text{weight of sample}
\]

\[
N (\%) = \frac{q \times N \times 14}{1000} \times 100\% = \alpha\%
\]

\[
M = \frac{147.1}{14} \times \alpha\%
\]

2.5 Solubility test water
Measurement of water solubility in the materials made using [7] methods. The filter paper was heated in an oven at a temperature of 105°C for 10 minutes, then cooled in a desiccator and weighed until a constant weight is found (a). The sample is weighed (initial weight) and then put on a 10 ml water
sample was dissolved in water and then filtered with filter paper of known weight. The filter paper was oven at 105°C for 3 hours. Then cooled filter paper in a desiccator and weighed it to obtain a constant weight (b). Solubility calculations done using the following formula:

\[
BK = (B - a)
\]

\[
D = \frac{(BA - BK)}{BA} \times 100\%
\]

2.6 Data analysis

Data obtained from the research results were processed using Analysis of Variance (ANOVA) to determine the effect of treatment given and followed by multiple range test Duncan (Duncan's Multiple Range Test), while the data is nonparametric form organoleptic tests analyzed using the Kruskal-Wallis test.

3. Result and discussion

3.1 Appearance test

Organoleptic test on this study aims to evaluate the preference level panelists to flavor products produced. Based on test results known that average acceptance panelists to color flavor in treatment P0, P1, P2, and P3 respectively 4.9; 6.3; 6 and 5.2. The results of non-parametric statistical tests Kruskal-Wallis to produce flavor powder color p-value 0.013 <0.05, which means that the critical value of treatment significant effect on the level of a powder flavor color.

In testing the aroma, outcome research indicates acceptance of the aroma flavor panelists in the control treatment P0 is 2.3 which means no fishy, while in treatment P1, P2 and P3 known that average revenue value of 3.3; 3.4; and 3.9, which means a bit fishy to neutral. The results of non-parametric statistical tests Kruskal-Wallis on the aroma flavor yield 0.004 p-value <0.05 critical value. This suggests treatment significant effect on the level of the powder flavor aroma preferences.

The test results for organoleptic acceptance taste or flavor in the control treatment known P0 2.1 which means no fish taste, while the treatment of P1, P2 and P3 known that average revenue value of 3.1; 3.7; and 3.9 which means rather tasteless fish until neutral. The results of non-parametric statistical tests using the Kruskal-Wallis to taste the flavor powder yield 0.000 p-value <0.05 critical value. This can mean treatment significant effect on the level of taste preferences in flavor powder.

Data extraction and organoleptic test results in flavor powder used to determine the flavor powder selected in this study. Data extraction and organoleptic testing flavor can be seen in Table 1.

| Treatment | The yield (%) | Color | Aroma | Flavor |
|-----------|---------------|-------|-------|--------|
| P0 (Control) | 6.18<sup>a</sup> | 4.9 | 2.3 | 2.1 |
| P1         | 7.23<sup>a</sup> | 6.3 | 3.3 | 3.1 |
| P2         | 9.98<sup>b</sup> | 6   | 3.4 | 3.7 |
| P3         | 12.03<sup>c</sup> | 5.2 | 3.9 | 3.9 |

Note: P0: Control, P1: Treatment with the concentration of the enzyme papain 2% (w/v), P2: Treatment with the concentration of the enzyme papain 4% (w/v), P3: Treatment with the concentration of the enzyme papain 6% (w/v). Notation different letters in the same column shows that there are significant differences among treatments (p <0.05)
Table 1 shows the different treatment of P2 and P3 real and has an average value of a higher yield than treatment P0 and P1. Control treatment or P0 produce the lowest yield of 6.18% and did not differ significantly with treatment P1 with the average value of the yield of 7.23%, while the flavor powder in P2 and P3 treatments resulted in yield of 9.98% and 12.03%, [8] mentions one factor that can increase the levels of material yield is the concentration of the dissolved substance and long time of extraction. Increasing the concentration of dissolved materials is directly proportional to the length of time of hydrolysis. The higher of concentration of the enzyme papain then the longer time required to reach the temperature hydrolysis.

The results showed that the addition of the enzyme papain treatment influence on organoleptic value of color flavor. Flavor generated in this study are yellow to yellow-brown. At the level of flavor known fondness for color P0 (4.9), P1 (6.3) P2 (6) and P3 (5.2). This means that flavor in treatment P0 and P3 somewhat out of favor until neutral, while the flavor on treatment rather preferred P1 and P2. The color change in flavor occurs after the drying process using the material which causes flavor oven until golden brown yellow. The color change in the drying process allegedly because Millard reaction is the reaction between reducing sugars with the primary amino acid group. In the treatment of P0 and P3 color acceptance known that the value lower than in the treatment of P1 and P2. The colors on the P0 treatment tends to amber while the P3 treatment paler color flavor making it less attractive, allegedly volume of different materials during the drying process by drying the same time provide a different maturity level that affect the color of flavor. This is in line with the statement [9] stated the level of intensity of the color depends on the drying time, drying temperature and chemical composition on the outer surface of the foodstuffs.

In the food industry, aroma assessment is important. Aroma testing can quickly provide feedback and assessment of product acceptance liked or disliked [10]. Hedonic quality test results on the flavor aroma showed the highest acceptance rate in a row in treatment P3 (3.9) followed by treatment P2 (3.4), P1 (3.2) and control P0 (2.3), which means somewhat flavorful fish until neutral. This result is not much different to the reception panelists to taste the flavor where the treatment with the highest value indicated in the treatment P3 (3.9) followed by treatment P2 (3.7), P1 (3.1) and control P0 (2.1). The average value of the aroma and flavor penerimana flavor increased along with the increase in the concentration of a given enzyme papain. The increase in value is anticipated by typical volatile components can be extracted in an optimal fish along with increased levels of the enzyme papain is used. Based on the test results yield and organoleptic tests are known flavors that are the treatments P2 and P3.

3.2 Selected flavor specifications
Flavor quality can be judged based on nutrient content and ease of use of the product. On the chosen flavor that treatment P2 and P3 (with the addition of the enzyme papain 4% and 6%) testing was done to determine the protein content of the nutritional value of such materials as well as physical testing of materials in water solubility test to determine the ability of the material in water solubility. Detailed description of flavor specifications selected in this treatment are presented in Table 2.
Table 2. Specifications of Selected Waste Materials Laundering Surimi Fish Meat lumatan

| Specification          | Value               | P3          | standard |
|------------------------|---------------------|-------------|----------|
| Appearance             |                     |             |          |
| Color                  | rather preferred    | Neutral     |          |
| Aroma                  | Rather fishy        | Neutral     |          |
| Flavor                 | Neutral             | Neutral     | -        |
| Chemistry              |                     |             |          |
| The protein content (%)| 15.62               | 11.39       | Min 7    |
| Physical               |                     |             |          |
| Solubility in water (%)| 96.5                | 93.9        | -        |
| Water content (%)      | -                   | -           | Max 4.0  |
| Figures microorganisms | Plate               | -           | Max 104  |

Flavor is a food additive that is important in a food product acceptance. Based on the results of research known hydrolysis process in manufacture of flavor effect on flavor characteristics. This is because the hydrolysis process uses heat and enzymes will produce some chemical reaction that can trigger the formation of flavor that pyrolysis of amino acids and peptides, caramelization of carbohydrates, especially sugar, degradation ribonucleotide, degradation of thiamine, the interaction of the amino acid or peptide with the sugar and the thermal degradation of lipids [11]. Hydrolysis process will break the polypeptide chain simultaneously. Group - carboxyl and primary amine in the amino acid will be bound to the amino acid. The type and sequence of amino acids in the peptide may contribute to the formation of the taste or flavor. Chain peptide containing glutamic acid residues will produce savory flavors.

The heating process resulting in a chemical reaction to form volatile compounds forming flavor. According Jayanti [9] volatile compounds that are responsible for the formation of flavor and aroma of fish are: derivatives of aldehydes, ketones, alcohols, amino acids and volatile fatty formed by the enzymatic process and aktifitas microorganisms. Winarno [12] added the acceptance of the flavor and aroma of a food is influenced by several factors, including the constituent chemical compounds in food, temperature, consistency and interaction with other chemicals.

Flavor caused by the presence of a compound flavor (flavoring agent) are present in very small amounts in food stuffs. Structural components in living cells which constitute the largest source of forming flavor are protein, fat, and carbohydrates Jayanti [9]. One compound on dissolved solids is an important component in creating flavor is a soluble protein. The protein content of flavor in P2 and P3 treatments known to 15.62% and 11.39%. These results have met the quality requirements of a flavoring taste set by SNI No. 1-4273-1996, wherein the flavoring has a protein content of at least 7%.

Flavor dissolved in a neutral solvent to facilitate its use. [13] mentions one common solvent used is water, it is necessary to note flavor solubility in water. Based on the results of research known flavor in treatment P2 has a water solubility of 96.5% higher than in Q3 is 93.9%. The solubility product in water flavor comparatively quite high. This is consistent with the statement [14] which mentions flavor ingredients generally have the nature of which has a high concentration, volatile, soluble or interact with water. Flavor has a high solubility values in water suspected because of the hydrolysis reaction is a reaction of proteins by enzymes breakdown proteins into small peptides. Hydrolysis process can open the bond formed by the interaction between hydrophobic groups, so it turns to
produce a hydrophilic carboxyl and amino end to bond with water. Results showed treatment P2 4% addition of the enzyme papain to produce flavor protein content (15.6%) and solubility of ingredients in water (96.6%) higher compared to the addition of the enzyme papain treatment P3 6% protein content (11.4%) and power ingredient solubility in water (93.9%). Based on these test results are known P2 treatment is the best treatment for protein content and a solubility in water higher. so it turns to produce a hydrophilic carboxyl and amino end to bond with water. Results showed treatment P2 4% addition of the enzyme papain to produce flavor protein content (15.6%) and solubility of ingredients in water (96.6%) higher compared to the addition of the enzyme papain treatment P3 6% protein content (11.4%) and power ingredient solubility in water (93.9%). Based on these test results are known P2 treatment is the best treatment for protein content and a solubility in water higher.

Product flavor of treatment P2 then in a further test to determine levels of glutamic acid in the ingredients. Amino acid glutamate is known to contribute produce savory flavors in food [15], [16] stated that glutamic acid, aspartic acid, glycine, and alanine is an amino acid that plays a role in improving the aroma (flavor enhancer) in fishery products. The content of glutamic acid in the flavor with a concentration of 4% papain enzyme known to 0.26%. Amino acid glutamic acid is an amino acid group R polar and negatively charged. Polar amino acid glutamic acid that can dissolve in water. Based on research [17] glutamic acid content in the waste water leaching material surimi fish meat lumatan sebasar 0.00167% whereas the form of concentrates from washing waste material lumatan meat surimi obtained using freez drying technology contains glutamic acid amounted to 5.49% Trilaksani [3]. This indicates that the processing of the sample can affect the levels of the amino acid glutamate in a material.

4. Conclusions

The use of the enzyme papain with different concentrations affect the yield and value of organoleptic flavor include color, aroma, and taste. Best papain enzyme concentrations in this study were treated P2 is the addition of 4% papain enzyme.

5. References

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