Formulation of vegetable seasoning made from raw material of coconut blondo protein hydrolysate

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Abstract. The commercial seasoning that sold in the market containing artificial Monosodium Glutamate (MSG) as a flavor enhancer. However, the excessive use of MSG can endanger the health of the body. Therefore, it requires an alternative non-MSG seasoning. The use of MSG can be replaced with natural ingredients from protein enzymatic such as blondo protein hydrolysate. This study was conducted to understand the nutritional content and glutamic acid of blondo protein hydrolysate, to find out the appropriate formulation in making seasoning of blondo protein hydrolysate, and to understand the acceptance level of seasoning that made of blondo protein hydrolysate. The result of the proximate test on blondo protein hydrolysate were containing 8.21% water, 35.62% protein, 22.18% fat, 3.25% ash, 20.40% carbohydrate, and 10.41% glutamic acid. Based on the scoring test, could be known that the highest score for the parameter of taste, color, and aroma was a formulation with addition blondo protein hydrolysate around 8-10%. Thus, it could be stated that the use of vegetable seasoning products as an MSG alternative was possible and acceptable for consumers.

1. Introduction

Nowadays, modern society prefers to consume the kind of instant food. One of them is using seasoning in food processing. Many commercial instant seasonings contain monosodium glutamate (MSG). However, the excessive use of MSG can endanger the health of the body. Therefore, requires an alternative non-MSG seasoning. The use of MSG can be replaced with a natural ingredient as a hydrolysis result of animal and vegetable enzymatic proteins [1]. Hydrolysis of enzymatic proteins is the breakdown of molecules from complex protein bond into amino acid using the help of protease enzyme [2]. The formation of amino acid, especially glutamic acid, it causes protein hydrolysates to have a good taste [3]. One of the results of enzymatic hydrolysis which has a high protein content is blondo protein hydrolysate. Blondo is a by-product of the process of making virgin coconut oil (VCO). Blondo is a white cream which has a protein content of 24.22% [4].

Making VCO using the cold pressed centrifugal method produces coconut oil yield of 68.84% and blondo yield of 30.97% [5]. This high amount of blondo yield can be used as a product that can increase the economic value of blondo. Blondo originating from the process of making VCO with cold pressed centrifugal method per 50 grams contains 30.32% water, 26.59% fat, and 16.38% protein. Blondo is then hydrolyzed by using the bromelain enzyme, resulting in blondo protein hydrolysate with a water content of 5.03%, fat content of 21.33%, protein content of 19.68%, and total yield of
19.52% [6]. This indicates that the blondo protein hydrolysate has great potential to be used as an alternative seasoning for vegetable substitutes for MSG.

2. Materials and Method

2.1. Characterisation of raw material
Test carried out on the raw material of blondo is a proximate test which includes tests of water (oven method), protein (Kjeldahl method), fat (Soxhlet method) [7], ash, and carbohydrate [8].

2.2. Preparation of raw materials
Dry coconut blondo was obtained from Kedai Sehat Jakarta Store and young pineapple hump was obtained from Blimbing Malang Market. The ingredients used in making vegetable seasoning formulation were white pepper, powder onion, powder garlic that was obtained from the Henipure Singosari Store, salt with "Cap Kapal" brand, and sugar with "Gulaku" brand.

2.3. Making of protein flour concentrate blondo
Dry blondo was wrapped in a filter cloth and pressed until the oil comes out. Then dried using a cabinet dryer at 50 °C for 3 hours. Then, it was crushed by using a blender and sifted using a 60-mesh sieve.

2.4. Making of bromelain enzymes
Young pineapple fruit was peeled and cleaned from the outer skin. Then, cut and took the hump part, then crushed using a juicer. Next, filtered using coarse filter paper.

2.5. Making of blondo protein hydrolysate
Blondo protein concentrate as much as 50 g then add 150 mL of aquades each, then stir. Heat the solvent over the hotplate by covering aluminum foil for 30 minutes, until a stable temperature of 50°C was obtained. After the temperature was reached, the solvent of blondo flour protein concentrate was added to the bromelain enzyme as much as 12.5 mL. Then hydrolyzed by heating and controlled the temperature of the material 50-55°C above the hotplate for 10 hours. After being hydrolyzed, the hydrolysate solvent was then heated at a temperature of 90-95 °C for 10 minutes to inactivate the enzyme used. Then the concentrated protein hydrolysate solvent was filtered using a filter cloth. The hydrolysis results were flattened in a baking sheet and dried by using a cabinet dryer at 70 °C for 5 hours. After drying, the blondo protein hydrolysate was crushed by using a blender and sifted using a 60 mesh sieve.

2.6. Testing of Glutamic Acid
Testing of glutamat acid on blondo protein hydrolysate using High Performance Liquid Chromatogram (HPLC) method. Chromatography conditions were in accordance with the Agilent method [17]. Briefly, the hydrolyzed samples and the norvaline-spiked amino acid standard solutions were derivatized with OPA. After derivatization, an amount equivalent to 2.5 μL of each sample was injected on a Zorbax Eclipse-AAA column, 5 μm, 150 × 4.6 mm (Agilent), at 40°C, with detection at λ = 338 nm. Mobile phase A was 40 mM NaH₂PO₄, adjusted to pH 7.8 with NaOH, while mobile phase B was acetonitrile/methanol/ water (45/45/10 v/v/v). The separation was obtained at a flow rate of 2 mL/min with a gradient program that allowed for 1.9 min at 0% B followed by a 16.3-min step that raised eluent B to 53%. Then, washing at 100% B and equilibration at 0% B was performed in a total analysis time of 26 min.

2.7. Making of vegetable seasoning
Additional seasoning consisted of 60% salt, 15% powder garlic, 10% red onion, 10% sugar, and 5% white pepper powder. Then, the ingredients were mixed until smooth. Furthermore, the raw material in
the form of blondo protein hydrolysate and additional spices was weighed based on the formula that has been designed, as follows:

| Material Composition          | Proportion of blondo protein hydrolysate and additional seasoning (%) |
|------------------------------|-----------------------------------------------------------------------|
|                              | F0       | F1       | F2       | F3       | F4       | F5       |
| Blondo protein hydrolysate   | 0        | 2        | 4        | 6        | 8        | 10       |
| Additional seasoning         | 100      | 98       | 96       | 94       | 92       | 90       |

2.8. Scoring test
The scoring test was conducted by giving an assessment of each sample based on the color, taste, and aroma of soup in accordance with predetermined attribute. Assessment of attribute for color parameter was 1 = very cloudy, 2 = cloudy, 3 = somewhat cloudy. The rating of the attribute for the taste parameter was 1 = very tasty, 2 = savory, 3 = rather savory. The rating of attribute for the aroma parameter was 1 = very typical, 2 = typical, 3 = rather typical.

2.9. Data analysis
Scoring test result data were analyzed by the Friedman test. If there was a significant difference (p≤0.05) between treatments, then it was followed by the Wilcoxon test.

3. Results and Discussion
3.1. Chemical characteristics of blondo
The main raw material used in making vegetable seasoning is blondo. Test carried out on the raw material of blondo is a proximate test which includes tests of water, protein, fat, ash, and carbohydrate level. The result of blondo raw material analysis can be seen in Table 2.

| Chemical Characteristics | Analysis Result (%) | Literature (%) [9] |
|--------------------------|---------------------|--------------------|
| Water                    | 9.09                | 3.52               |
| Protein                  | 19.95               | 19.54              |
| Fat                      | 34.51               | 52.64              |
| Ash                      | 5.00                | 2.51               |
| Carbohydrate             | 31.45               | 21.78              |

Based on Table 2, it shows that the chemical content of the material between the analysis result and literature is different. The difference in chemical characteristics of blondo from the result of analysis and literature is caused by differences in the type and age of coconut harvest used in making VCO and the different methods used in making VCO, so the resulting blondo is also different. Blondo which is used in this study comes from the processing of the VCO centrifugal cold-pressed method, while the blondo used in the literature comes from the processing of the VCO acidification method.

3.2. Chemical characteristics of blondo protein hydrolysate
Blondo is then hydrolyzed to break down proteins into amino acid to produce blondo protein hydrolysate which are easier for the body to digest. Test performed on blondo protein hydrolysate
includes tests of water, protein, fat, ash, carbohydrate, and glutamic acid levels. The comparison of the analysis result of the chemical characteristics of blondo and blondo protein hydrolysate can be seen in Table 3.

**Table 3. Result of analysis of the chemical characteristics of blondo and hydrolysis of protein blondo**

| Chemical Characteristics | Blondo    | Blondo Protein Hydrolysate |
|--------------------------|-----------|----------------------------|
| Water                    | 9.09      | 8.21                       |
| Protein                  | 19.95     | 35.62                      |
| Fat                      | 34.51     | 22.18                      |
| Ash                      | 5.00      | 3.25                       |
| Carbohydrate             | 31.45     | 20.40                      |

Based on Table 3, it shows that the protein of blondo hydrolysate contains 8.21% lower than blondo, which is 9.09%. This is due to the drying process which aims to extend the product shelf life [9]. Blondo protein hydrolysate contains 35.62% protein, while blondo contains protein of 19.95%. During the hydrolysis process, protein breaks down into peptide bonds which can increase polar amine and carboxyl groups so that they can increase protein solubility [10]. The efficiency of blondo protein extraction is influenced by protein solubility, comparison of raw materials and solvent, temperature, and extraction time [11]. The fat content of the blondo protein hydrolysate is 22.18% which decreases by about 12% from blondo, which is 34.51%. Decreasing fat content in the product is caused by the pressing process of blondo. To separate oil from materials containing oil content above 10%, it can be done by pressing using screw press [12]. The separation of oil on blondo can be done by pressing then followed by extraction using hexane solvent [10]. Blondo protein hydrolysate has an ash content of 5.00%, while the raw material for blondo is 3.25%. Ash content is related to the content of inorganic minerals in these foods. The decrease in ash content is caused by mineral salt that dissolves in water during boiling or hydrolysis [13]. The carbohydrate content of the blondo protein hydrolysate changes when comparing to the raw material of blondo, which is equal to 31.45%, decreases to 20.40%. The decrease in the value of carbohydrate content is caused by heating at high temperatures which results in carbohydrate molecules becoming damaged [15].

3.3. Glutamic acid

The highest nonessential amino acid contained in the protein blondo hydrolysate is glutamic acid with a percentage of 10.37%. Monosodium Glutamate (MSG) is approximately 78 percent free glutamic acid, 21 percent sodium, and up to 1 percent contaminants [16]. Glutamic acid is a constituent of proteins found in various vegetables, meat, fish and breast milk. Glutamic acid is more contained in vegetable protein, which is around 40%, whereas in animal protein it only contains 11-22% glutamic acid [15]. Glutamate performs several important functions in metabolic processes in the body, namely substances for protein synthesis, transamination pairs with α-ketoglutarate, glutamine precursors, and neurotransmitters [15].

3.4. Scoring test

a. Taste

Based on the analysis of taste result, it shows that the significance value is 0.00 <significance level α = 0.05 and the calculated Chi-square value is 72.93> Chi-square table of 11.07. Figure 1 shows that the taste score values of vegetable seasoning tend to increase from formulas F0 to F5. The perception of food taste can be influenced by internal factors such as the sensitivity of the tongue papilla and external factors such as chemical compound, temperature, concentration and interaction of other taste components [18]. While the highest taste score, which is found in the F4 formula, is the highest value that is equal to 2.57 with the very tasty specification. However, the F4 formulation is not significantly
different from the F5 formulation, so it can be stated that the taste in vegetable seasoning soup product is affected by the addition of blondo protein hydrolysate about 8-10%. The greater the proportion of blondo protein hydrolysate, the more delicious the taste of the soup. Addition of protein hydrolysate in food product processing will increase the savory taste [19]. Protein hydrolysis will create changes to the taste of food caused by the formation of short chain peptides and free amino acid which play a role in the formation of savory flavors [5]. Savory taste or umami in food comes from the presence of amino acids in the form of glutamic acid [20].

b. Color
Based on the analysis result of color scoring, it shows that the significance value is 0.000 < significance level α = 0.05 and the calculated Chi-square value is 85.411 > Chi-square table of 11.070. Figure 2 shows that the color score value of vegetable seasoning has increased from formulation F0 to F5. The color score value in treatment F5 is the highest value that is equal to 2.70 with the very turbid specification. However, the F4 formulation is not significantly different from the F5 formulation, so it can be stated that the taste in vegetable seasoning soup product is affected by the addition of blondo protein hydrolysate about 8-10%. This indicates that the color of the vegetable seasoning soup product is influenced by the addition of blondo protein hydrolysate. The greater the proportion of blondo protein hydrolysate, the more the color of the soup becomes cloudy. Additional seasoning in the form of spices is naturally brownish/dark so they can affect the color of the food during processing [19]. The brown color of natural seasoning is derived from the melanoidin compound from the Maillard reaction. The reaction that occurs between hydroxyl groups in sugar with amino groups from amino acid during the drying process will form compounds that produce brown color [22].

c. Aroma
Based on the analysis result of aroma scoring values using the Friedman test, it shows that the significance value is 0.000 < significance level = 0.05 and the Chi-square value of 55.604 > Chi-square
table of 11.07. This indicated that the difference in the proportion of blondo protein hydrolysate has a significant effect on the aroma of vegetable seasoning. Figure 3 shows that the scent score on vegetable seasoning increases from formula F0 to formula F5. Proteins hydrolysate contain volatile amino acid. This type of amino acid can produce hydrogen sulfide and other sulfur volatile compounds that can form fleshy aromas in food [22]. Scent score value in treatment F5 is the highest value that is equal to 2.57 with the very typical specification. However, the F4 formulation is not significantly different from the F5 formulation, so it can be stated that the taste in vegetable seasoning soup product is affected by the addition of blondo protein hydrolysate about 8-10%. This indicates that the aroma of vegetable seasoning soup product is influenced by the addition of blondo protein hydrolysate. The greater the proportion of blondo protein hydrolysate, the more distinctive aroma of the soup. The Maillard reaction causes a distinctive flavor change in the food product. The heating process also degrades volatile compounds to produce a large number of aroma components. The type of aroma produced depends on the special combination of fat, amino acid, and sugar found in food ingredient [23].

4. Conclusions
Blondo protein hydrolysate can be used as a vegetable seasoning which is an alternative non-MSG seasoning. The vegetable seasoning formulated from coconut blondo contains nutrient in the form of water 9.09%, protein 19.95%, fat 34.51%, ash 5.00%, carbohydrate 31.45%. On the other hand, the vegetable seasoning formulated from blondo protein hydrolysate is 8.21% water, 35.62% protein, 22.18% fat, 3.25% ash, and 20.40% carbohydrate. Glutamic acid contained in blondo protein hydrolysate is about 10.37%. Based on the scoring test, it is found that the highest score for taste, aroma, and color parameter is the formulation with the addition of blondo protein hydrolysate around 10%. Therefore, it can be stated that the use of vegetable seasoning product as an alternative to MSG is possible and acceptable to consumers.

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