The substitution effect of bone fish flour milkfish (Chanos chanos) physical and chemical characteristics of cookies

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Abstract. Confectionery is a relatively dry food grilled foods have lasting power is high, so it can be stored for a long time and is easy to carry while traveling, due to volume and weighs a relatively light due to the drying process. Physical and chemical characteristics of biscuits are factors that affect the quality of the final product. The content of the fish bone flour, namely: calcium 39.24%, 13.66% phosphorus, water content 5.60%, ash 81.13% bb bb 0.76% protein and 3.05% fat. This study aims to determine the effect of substitution of fish bone flour to the physical and chemical characteristics of the biscuit. This research was carried out experimentally with a completely randomized design (CRD) with 4 treatments and 5 replications. The treatment is given in the form of increasing concentration of 0% (as a control), 10%, 20% and 30%. Based on the research of fish flour of fish bones in the manufacture of biscuits to give effect to the chemical characteristics of the biscuit, but does not give effect to the physical characteristics of biscuits. The best treatment is found in treatment P3 concentration of 30%.

1. Introduction
Malnutrition is divided into two classes, the first class is primary malnutrition, for example in deficiencies of specific essential nutrients, such as deficiencies in vitamins, calcium and phosphorus, and patient experiences symptoms of scurvy. The second class of malnutrition is secondary malnutrition, for example caused by impaired absorption of nutrients or disorders of nutrient metabolism [1]. Therefore, we need food intake with nutritional value that can meet all nutritional needs. Fish flour is a solid product that is produced by removing most of the water and part or all of the fat in fish or fish waste [2]. Fish flour products contain quite high calcium and phosphorus so that they have the potential to meet calcium intake. Until now, the use of fish flour has not been done optimally. The main use of fish flour is still a mixture of animal feed [3].

Milkfish bone waste has the potential to be used for flour substitution for food. One of the centers of milkfish filleting business that is located in Sidoarjo, can produce milkfish bone waste up to 15 kg/day. In one month, the milkfish bone waste reaches 450 kg. The use of fish flour as a substitute for wheat flour in biscuits is a promising alternative, especially in terms of the quality of nutrients produced [4]. Biscuits are practical food because they can be eaten anytime and with good packaging, biscuits have a relatively long shelf life [5].

2. Material and methods
2.1. Place and time of experiment
This research was conducted from June to July 2016 at the Processing Laboratory, Department of Nutrition, Faculty of Public Health, Universitas Airlangga, Surabaya.

2.2. Tools and material
The materials used in this study consist of ingredients for the manufacture of biscuits, namely flour, milkfish bone flour, egg yolk, butter, sugar, milk and baking powder.

Tools used in this study consisted of tools for making fish bone flour, biscuits and analysis activity. The tools used in the manufacture of fishbone flour are gas stoves, pans, plastic basins, analytical scales,
blenders, cutting boards, ovens while the tools for making biscuits include pans, trays, molds and ovens. Tools for analytical purposes such as plates, scales, cruss pliers, bunsen ovens, electric furnaces, etc.

2.3. Work procedure

2.3.1. Preparation of raw material

The selection of raw materials and the collection of raw materials began in early February in several places such as milkfish filleting industry, traditional markets and grocery stores. The selection of raw materials, especially milkfish bone waste, is based on the texture and physical appearance of the material. The equipments used are bone flour producing machine and analysis equipment for biscuits given the addition of bone flour. There are also surimi making equipment such as; knife, chopper, meat crusher, basin etc.

2.3.2. Producing of milkfish bone flour

Making of biscuits begins with the process of making bone flour. First, milkfish bones were washed with water, then boiled for 30 minutes at a temperature of 100°C. Then, separate the excess meat from the bones. After that, the milkfish bones were heated in an autoclave at a temperature of 121°C for 2 hours and then dried using an oven at a temperature of 120°C for 35 minutes and then milled using a blender.

2.3.3. Making biscuits with addition of bone flour

The biscuit-making process in general consists of mixing, shaping and baking. The ingredients used in the manufacture of biscuits consist of 400 grams of wheat flour, 200 grams of milkfish bone flour, 4 grams of egg yolk, 200 grams of butter, 120 grams of sugar, 16 grams of milk, 1 gram of baking powder [6]. In this study, the mixing method used is single-stage, which means, all ingredients are mixed together and stirred together. This method has similarities with the all-in method, in this method all ingredients are mixed together. This method is faster and the resulting dough is denser and harder than the dough in the cream method [7]. The dough obtained is then shaped according to the desired shape and size. The biscuit dough is formed into sheets and cut into pieces with a cutting knife or a biscuit mold. The dough that has been printed is then baked in the oven. In general, the temperature for baking biscuits is between 218-232°C within 15-20 minutes [8]. The concentrations given were 0%, 10%, 20% and 30%, respectively. The parameters observed in this study were the content of calcium, organoleptic and acceptability of biscuits.

3. Results and discussion

Organoleptic tests conducted on flavor, aroma, color and texture of biscuits were carried out by 25 untrained panelists. The results of the organoleptic test for each treatment can be seen in Table 1.

| Table 1. Organoleptic test mean value |
|--------------------------------------|
| Parameter | P0 (TB 0%) | P1 (TB 10%) | P2 (TB 20%) | P3 (TB 30%) |
| Flavor    | 3.8        | 3.56        | 3.2         | 3.48        |
| Aroma     | 3.6        | 3.28        | 3.08        | 3.36        |
| Color     | 3.76       | 3.52        | 2.92        | 3.36        |
| Texture   | 3.76       | 3.56        | 3.32        | 3.28        |
| Acceptibility | 3.92   | 3.64        | 3.32        | 3.44        |

Note: P0 = Without substitution of milkfish flour 0% (control), P1 = With substitution of 10% milkfish bone flour, P2 = With substitution of 20% milkfish bone flour, P3 = With substitution of 30% milkfish bone flour. TB = Milkfish flour.

Variety of tests show that biscuits without milkfish bone meal substitution (control) are the highest average value in all parameters. The Kruskal Wallis analysis of aroma, flavor and texture parameters did not show a significant difference (p≥0.05). Meanwhile, the analysis on color parameter showed
significantly different result ($p<0.05$). Based on the results of further analysis of variance, it showed that the substitution of milkfish bone flour on wheat flour had a significant effect ($P <0.05$) on the ash content of biscuits. The results of the variance test also showed that the mean value of ash content showed significantly different results for each treatment. Biscuits treated with P3 (milkfish flour concentration 30%) were significantly different from treatment P2 (milkfish flour concentration 20%), P2 was significantly different from P1 concentration (milkfish flour concentration 10%) and P1 was significantly different from P0 (control).

**Table 2. The average value of the ash content test**

| Treatment | Average of Ash Content ± SD (%) |
|-----------|---------------------------------|
| P0        | 1.07$^d$ ±0.02                  |
| P1        | 3.79$^a$ ±0.04                  |
| P2        | 6.62$^b$ ±0.06                  |
| P3        | 9.27$^a$ ±0.07                  |

Note: P0 = Without substitution of milkfish bone flour 0% (control), P1 = With substitution of 10% milkfish bone flour, P2 = With substitution of 20% milkfish bone flour, P3 = With substitution of 30% milkfish bone flour. TB = milkfish bone flour.

The water content test conducted with analysis of variance showed significantly different results ($p \geq 0.05$) for each treatment. Biscuits with treatment P1 (concentration of milkfish flour 10%) were significantly different from treatment P3 (concentration of milkfish flour 30%) and P0 (control). Meanwhile, P3 and P0 were also significantly different from P2 (milkfish flour concentration 20%). The results of the water content in each treatment can be seen in Table 3.

**Table 3. Average value of moisture content**

| Treatment | Average of Water Content ± SD (%) |
|-----------|----------------------------------|
| P0        | 6.22$^b$ ±0.10                   |
| P1        | 6.78$^a$ ±0.19                   |
| P2        | 5.73$^a$ ±0.28                   |
| P3        | 6.26$^b$ ±0.33                   |

Note: P0 = Without substitution of milkfish bone flour 0% (control), P1 = With substitution of 10% milkfish bone flour, P2 = With substitution of 20% milkfish bone flour, P3 = With substitution of 30% milkfish bone flour.

The result of fat test showed that the highest value was in the control treatment which was 16.68%. This value is not significantly different from treatment 2 which shows the number of 15.56%. The value of the two is very significantly different from treatment 1 and 3. The value in treatment 3 showed a value of 14.30% and treatment 1 showed value of 14.08%.

**Table 4. Average value of fat content**

| Treatment | Average Fat Content ± SD (%) |
|-----------|-----------------------------|
| P0        | 16.68$^a$ ±0.79             |
| P1        | 14.08$^b$ ±0.63             |
| P2        | 15.56$^a$ ±0.70             |
| P3        | 14.30$^b$ ±0.88             |

Note: P0 = Without substitution of milkfish bone flour 0% (kontrol), P1 = With substitution of 10% milkfish bone flour, P2 = With substitution of 20% milkfish bone flour, P3 = With substitution of 30% milkfish bone flour.

The highest protein yield was found in the P3 treatment which was 9.23% while the P2 and P1 treatments were 9.04% and 8.84%, respectively. The value of the three treatments is significantly different from
treatment 0 which indicates a value of 8.08% every treatment. The results of the protein yield test for each treatment can be seen in Table 5.

| Treatment | Average Protein ± SD (%) |
|-----------|--------------------------|
| P0        | 8.08 ± 0.21              |
| P1        | 8.84 ± 0.43              |
| P2        | 9.04 ± 0.33              |
| P3        | 9.23 ± 0.34              |

Note: P0 = Without substitution of milkfish bone flour 0% (kontrol), P1 = With substitution of 10% milkfish bone flour, P2 = With substitution of 20% milkfish bone flour, P3 = With substitution of 30% milkfish bone flour.

The physical test of biscuits was carried out by observing the components of flavor, aroma, color and texture of the biscuits in each treatment. Based on the Kruskal Wallis test results, the addition of milkfish bone flour did not significantly affect the texture, flavor and aroma of the biscuits. The level of preference for the panelists towards texture, flavor and aroma was at the normal level, presumably because at the kneading stage they still used conventional methods. This process resulted in the appearance of the biscuits that were not homogeneous. In addition, the roasting process does not use a stable temperature so that the resulting color is not uniform. The roasting should be at a temperature of 160°C for 5 minutes, or a temperature of 135°C for 20 minutes, so that the resulting product does not burn [9]. The treatment of increasing the concentration of milkfish bone flour did not have a significant effect on the aroma, texture and flavor of the total biscuits.

The mean scores on the taste parameter ranged between 3.2 and 3.8. This shows that biscuits with a sweet taste and a little fishy are preferred by the panelists. Factors that affect taste are closely related to chemical compounds, temperature, and the interactions of other components. Various chemical compounds give different flavors. Sour taste is caused by protons, salty taste is produced by inorganic salts, sweet taste is also caused by aliphatic organic compounds and bitter taste is caused by alkaloids. Interaction with other components can affect the value of a product's taste

The average scores on aroma parameters ranged between 3.08 and 3.6. Based on this value, it shows that the criteria for biscuits with a slight vanilla aroma and specific fish aroma are preferred by panelists. The aroma component determines the quality and taste of a food ingredient. Protein in a food ingredient can affect the aroma of food. On heating, the protein in food will change and form compounds with other ingredients, for example with amino acids that result from changing proteins with reducing sugars that form food aromas. The aroma of food is recognized in the form of steam

The heating process by roasting the biscuits can cause protein denaturation and increase digestibility. Roasting can also cause free amino acids to bind with carboxyl groups of reducing sugars such as fructose, lactose, and maltose to form a Maillard nonenzymatic reaction. The Maillard reaction is responsible for forming the aroma and taste of the product through the roasting process [5]. The average scores on the color parameter range from 2.92 and 3.76. Based on this value, it shows that the criteria for biscuits with a slightly dull brown color are preferred by the panelists. In the color component, the brown color changes in the flour due to the heating process during roasting. This brown color is caused by the Maillard reaction. The Maillard reaction is a reaction that involves reducing sugars and also the amine group from the protein due to heating and produces a new brown color, namely melanoidin [10].

The mean value of the texture parameter ranges between 3.28 and 3.76. Based on this value, it shows that the criteria for biscuits with a hard, dense and slightly dry texture are preferred by panelists. In texture testing with Kruskal Wallis analysis, there was no significant difference in each treatment. Texture plays an important role in the sensory of a product [11]. Texture is influenced by the water content in the material, if the water content is high it will cause the material to be less dry so that it is difficult to destroy, thus it will produce coarse particles.

The average response of the panelists to the color of biscuit was normal (neutral), this is presumably
because the biscuit-making process used ingredients in the form of wheat flour, sugar and additional milkfish bone flour which made the resulting begae cake to be less bright. The panelists’ preference for biscuit color was influenced by the addition of milkfish bone flour. Calcium particles will reduce the brightness of the resulting product. Besides, the brown color of the biscuits is thought to be due to the Maillard reaction, so that when the roasting process occurs, a reaction will occur between carbohydrates, especially reducing sugars, and the primary amine acid groups found in the material so that it will produce a brown material called melanoidin.

Based on the testing of biscuit’s ash content with the addition of milkfish bone flour using analysis of variance (ANOVA), resulted in a significant effect on each treatment. The water content in the selected biscuits with the milkfish bone flour substitution of 20% (P2) resulted in an average number of 5.73%. The maximum moisture content that is allowed in biscuits is 5% [12]. The water content value of biscuits produced by the addition of milkfish bone flour met SNI standards. Biscuits with the addition of 20% bone meal showed lower number of water content when compared to biscuits with other treatments.

The water content in the biscuits is reduced due the shape of the water molecules in the biscuits, which is free water that is prone to loss due to evaporation along with the baking process. This free water component is located in the inter-granular space between the cells and the pores of the food material so that the free water can affect the process of food damage, for example microbiological, chemical and enzymatic processes. The substitution of milkfish bone flour affects the water components contained in the material. The water then will be bound by Ca ++ particles contained in fish bone flour, causing the water content to reduce. With the addition of tuna fish bone flour, Ca ++ particles will bind OH - particles which are part of the water elements or H2O so that water content decreases along with the addition of milkfish bone meal.

The value of water content can be used as a parameter for fat and protein content, because materials that have low water content tend to have high levels of fat and protein [13]. Water content plays a role in determining the freshness and acceptability of a product. The lower the water content value of a material, the higher the quality of the material because it can reduce the media for microbial growth and can reduce the quality of flour. Biscuit products in P2 treatment of 5.73% met the biscuit moisture content standard, namely a maximum of 5% [12]. The difference in levels that occurs is mostly influenced by the heating process in each biscuit treatment.

Many water molecules are evaporated from the biscuit material while roasting stage. Different roasting conditions will affect the formation of structures in which the amount of water that must be removed depends on the richness of the ingredient formulation. The baking of biscuits also results in a loss of moisture, which is followed by a transfer of moisture to the surface which is continuously lost to the oven hot environment. The water content value that is too low causes the biscuits to have a bitter taste and a dark appearance, but if they are too high, the structure will not become crispy, can easily be fractured (checking), and changes in flavor during storage will occur more quickly. The high proximate composition of protein, fat and ash values can be caused by water loss so that nutrients are concentrated during processing [14].

Based on the testing of biscuit’s ash content with the addition of the concentration of milkfish bone flour using analysis of variance (ANOVA), resulted in a significant effect on each treatment. The resulting ash content tended to increase in each treatment along with the addition of the milkfish bone flour concentration. This condition can occur because of the additional minerals in fish bone meal that are added in the material [6]. The value of high ash content is due to the main components of the bone which are minerals. The bones contain living cells and intracellular matrix in the form of mineral salts. Mineral salt is a component consisting of calcium phosphate as much as 80% and the rest consists of calcium carbonate and magnesium phosphate.

The ash content of a material describes the amount of minerals that do not burn into a volatile substance. The greater the ash content of a food ingredient, the higher the minerals contained in the food. Ash content that is too high can cause the dough to be resistant to expand. Biscuit products in treatment P0 with a value of 1.07% is in accordance with the standard of biscuit ash content, namely a maximum
of 1.6% [12]. The difference in levels that occurs is mostly influenced by the heating process in each biscuit treatment.

Based on testing the fat content in biscuits with the addition of the concentration of milkfish bone meal with analysis of variance (ANOVA) showed that the fat content of the biscuits had an effect on each treatment. The resulting fat value tends to decrease with the amount of bone meal concentration given. The processing of food will damage the fat contained in it. The degree of fat breakdown varies widely depending on the temperature used and the length of processing time. The higher the temperature used, the more intense the fat breakdown will be. The decrease in fat content after boiling is due to the nature of the fat which is not heat resistant, during the cooking process the fat melts and even evaporates (volatile) into other components such as flavor. The low value of fat content is also possible because the water content in the treatment decreases. The value of water content has a strong correlation with fat content [6]. Loss of fat and water content can occur due to denaturation of protein in the tissue in a level that can lead to decrease in water binding capacity and protein emulsification properties. The cutting of bone during the manufacture of bone flour also helps release fat and water as it also increases the surface area of the material in contact with the heat during the cooking stage. Biscuit products in all treatments met the fat standard, namely a minimum of 9.5% [12].

Based on the examining the protein value of biscuits with the addition of the concentration of milkfish bone flour with analysis of variance (ANOVA), showed an effect on each treatment. The average protein content produced in the selected products was 6.6%. Meanwhile, the minimum protein content for biscuits is 9% [12]. The protein content of biscuits produced in the study was still less than the minimum SNI requirements. The mean protein value increases with the addition of flour concentration. The protein content is influenced by the water content that is lost from the material. The high protein content in the material can be due to the decreasing of moisture content of the material due to the drying process. The lower the water content, the higher the value of crude protein in the material. Biscuits in P2 and P3 treatment have a value of 9.04% and 9.23% where these values have met the standard, namely a minimum of 9% [12].

Biscuits baking can cause reactions in proteins including denaturation, loss of enzyme activity, color change, cross-linking, breaking of peptide bonds, and the formation of sensory active compounds. This reaction is influenced by temperature and heating time, pH, the presence of oxidizing agents, antioxidants, radicals, and other active compounds, especially carbonyl compounds.

Most food protein is denatured when heated at moderate temperatures (60-90 °C) for one hour or less. Denaturation is a change in protein structure where in the fully denatured state, so only the primary structure of the protein remains, it cause the protein to no longer has a secondary, tertiary and quaternary structure. Excessive denaturation of proteins can lead to insolubilization which can affect the functional properties of proteins depending on their solubility.

4. Conclusion
Substitution of milkfish bone flour with a concentration of 10%, 20% and 30% affects the chemical characteristics of biscuits. The best treatment was obtained in the P1 treatment (10% substitution of milkfish flour) with an ash content of 3.79%, a water content of 6.78%, 14.08% fat and 8.84% protein. Treatment P1 (substitution of milkfish flour 10%) has physical characteristics, namely a slightly dull brown color, hard texture, dense and slightly dry, slightly vanilla aroma and sweet taste and not fishy.

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