

Improving Macro Nutrition Content on Nutritional Biscuits with Substitution of Tilapia Fish Flour, Sardine Fish Flour, and Red Rice Flour

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ABSTRACT—*The objective of the research: to study the macro nutrient content of biscuits with substitution of tilapia fish flour, sardine fish flour, and red rice flour. Method: experimental research, the study was conducted in March – June 2017 at Culinary Laboratory Universitas Negeri Makassar and Biophysics Laboratory, Faculty of Public Health, Universitas Hasanuddin. Main ingredients: 1 biscuit without substitution (F0) and 7 biscuits with substitution: tilapia fish flour substituted biscuit (F1), sardine fish flour substituted biscuit (F2), red rice flour substituted biscuit (F3), tilapia and sardine fish flour substituted biscuit (F4), tilapia fish flour and red rice flour substituted biscuit (F5), sardine fish flour and red rice flour substituted biscuit (F6), and tilapia and sardine fish flour and red rice flour substituted biscuit (F7). Data collection by proximate analysis: water content AOAC (2012) 930.15, ash content AOAC (2012) 942.05, fat content AOAC (2012) 991.36, protein content IK.LP-04.3-LT-1.0, carbohydrate content IK.LP-04.3-LT-1.0, and presented in histograms. The Results of Nutrient Content in the biscuits with substitution of tilapia fish flour, sardine fish flour, and red rice flour are respectively energy, protein, fat, carbohydrate, water, and ashes: F0: 476.03kcal; 7.72g; 17.43g; 72.07g; 2.03g; 0.75g. F1: 500.33Kcal; 13.59g; 24.77g; 55.76g; 4.48g; 1.4g. F2: 490.65Kcal; 11.01g; 21.49g; 63.3g; 3.03g; 1.17g. F3: 507.46Kcal; 11.68g; 24.46g; 60.15g; 2.52g; 1.19g. F4: 478.78Kcal; 11.05g; 19.3g; 65.22g; 3.1g; 1.33g. F5: 492.3Kcal; 11.25g; 21.75g; 62.89g; 2.88g; 1.23g. F6: 483.06Kcal; 10.03g; 20.43g; 64.77g; 3.31g; 1.46g. F7: 478.11Kcal; 7.8g; 20.23g; 66.21g; 3.94g; 1.82g.*

Keywords--- Biscuit, Macro nutrient content, Proximate analysis

1. INTRODUCTION

Nutrition depends on the educational achievement and how long the education is. Children with the nutritional problem will face problems in growth and development, and may become less educated adult, poor, unhealthy, and more vulnerable to illnesses [1]. The cohort study Steckel concluded the small size of babies and stunting are closely related to the height of adults, decline of body fat mass, short school period, and decline in intellectual function, which will be resulted in the low weight of newborns [2].

The nutrition problems for school children is optimized and handled through diversification of additional food formula development such as biscuit, taking into account the aspects of nutrition, health benefit, absorption, endurance, and superior local food resources such as freshwater fish, sea fish, and red rice. Fish is one of the ingredients that is less favored by the public due to the fishy scent and taste, and the number of bones in fish, even though the nutrient content of fish is very high, especially in protein and calcium [3][4][5]. Another potential material with limited use is red rice. Red rice contains antioxidants to prevent the occurrence of various degenerative diseases. Currently, the public have not consumed red rice due to the low characteristic of red rice compared to white rice [6][7].

The nutrition problems for school children is optimized and handled through diversification of additional food formula development such as biscuit, taking into account the aspects of nutrition, health benefit, absorption, endurance, and superior local food resources such as freshwater fish, sea fish, and red rice. Fish is one of the ingredients that is less favored by the public due to the fishy scent and taste, and the number of bones in fish, even though the nutrient content of fish is very high, especially in protein and calcium [3][4][5]. Another potential material with limited use is red rice.

Red rice contains antioxidants to prevent the occurrence of various degenerative diseases. Currently, the public have not consumed red rice due to the low characteristic of red rice compared to white rice [6]

Based on that fact, the formulation of supplement food with high nutrient by utilizing fish and red rice in the form of biscuits. Biscuit has the advantage of its small size, long shelf life, and well received by the community. To date, the manufactured biscuits are not enriched with local food ingredients that contain high nutrients such as fish and red rice.

The research is focused on the utilization of fish and red rice in the biscuit production, with the expectation that it will be well received. The more the public like the biscuit product, the more the nutrition of biscuit is absorbed. Based on this, it is urgent to conduct research to study the biscuit production process by utilizing those three ingredients. Therefore, the objective of this research is to produce biscuit formula with additional tilapia fish flour and red rice flour.

The objective of this study is to review the macro nutrition content on nutritious biscuits with the substitution of tilapia fish flour, sardine fish flour, and red rice flour.

2. MATERIALS AND METHODS

2.1. Place and Time of Study

The study is an experimental study conducted in March until June 2017. The biscuit formulation experiment is conducted at Culinary Laboratory Universitas Negeri Makassar and Biophysics Laboratory, Faculty of Public Health, Universitas Hasanuddin.

2.2. Ingredients and Equipment

The main ingredients of this research are for nutritional biscuit with the base of tilapia (*Oreochromis mossambicus*) fish flour, sardine (*Sardinella aurita*) fish flour, and red rice (*Oryza nivara*) flour, which include wheat flour, tapioca flour, cornstarch, tilapia fish flour, red rice flour, margarine, egg yolk, refined sugar, and brown dye. The ingredients in analyzing the products include a mix of selenium, concentrated H₂SO₄, boric acid, HCl, hexane, and Whatman filter paper no. 93, aquades.

The equipment for biscuit production are: spoons, spatulas, knives, basins, pots, pans, blenders, mixers, biscuit molds, flour sifters, stoves, steaming tools, scales, and ovens. The equipment for analysis are: analytic scales, clamp glass tools (regular Erlenmeyer, Erlenmeyer, measuring cup, glass cup, measuring flask), volumetric pipet, micro pipet, Soxhlet extraction equipment, Kjeldahl flask, buret, vertical coolers, desiccators, ovens, spectrophotometry, reaction tubes, Petri dishes, autoclaves, incubator, humidity chamber, vortex, Bunsen, and HPLC.

2.3. Data collection and data analysis

Data is collected by measuring the macro nutrition content of the biscuit produced, based on each proximal of biscuit formula produced. Proximate Analysis. Chemical quality testing is one of the most important factors in determining the quality of foodstuffs. Chemical composition analysis (water content analysis, ash, protein, fat, and carbohydrate, acid fiber, mineral, etc.) is very important in order to know the nutritious value of a product [8].

The proximate analysis includes: **Water content analysis** with direct method of water absorption from ingredients with the assistance of oven, desiccation, distillation, extraction, and other physic-chemistry technics. The water content is measured by scaling or another direct method; **Ash content analysis**. The direct method is determined by weighing the residue of organic matter combustion at 550°C temperature; **Protein Content Analysis** estimates the protein that can be utilized by the human's body. The determination of total N in foodstuffs is often used as a predictor of protein content, as the method is based on the reaction that changed N into ammonium sulfate and in the end, ammonia. Further, ammonia is distilled and filtrated; **Fat Content Analysis**. The soxhlet extraction method uses fat that is extracted with organic solvent, then the solvent is evaporated and the fat is weighed. The dietary fat analysis is measured by Gas Chromatography (GC) to figure out the composition of fatty acid; **Carbohydrate Content Analysis**. Calculation analysis is conducted by reducing ingredients that are tested with the amount of water, ashes, fat, and protein. Proximate analysis: water content AOAC (2012) 930.15, ash content AOAC (2012) 942.05, fat content AOAC (2012) 991.36, protein content IK.LP-04.5-LT-1.0, carbohydrate content IK.LP-04.3-LT-1.0. Data analysis uses descriptive histogram of macro nutritious biscuit

3. RESULTS AND DISCUSSIONS

3.1. Production phase

The process of making biscuits based on tilapia fish flour and red rice flour begins with the preparation of ingredients and equipment used. Then, the ingredients are weighed according to the formula. The ingredients are mixed into biscuit dough, weighing 6 grams or 12 grams per ingredients. Further, molding each piece, baking/oven until cooked, and then colling and packing. The biscuit making process can be seen in Figure 1.

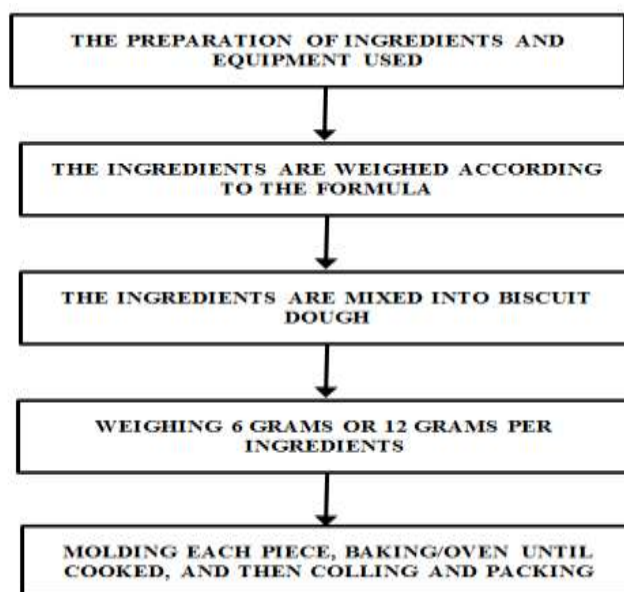


Figure 1. Biscuit producing process diagram

3.2. Formulation

The biscuit formulations are all formulation produced in the process of tilapia fish flour and red rice flour based nutritious biscuits, and referred to Widodo et al. 2017 . The biscuit formulation is presented in this Table 1

Table 1. Nutritious biscuit formula with basis of tilapia fish flour, sardine flour, and red rice flour per 100 grams of dough

Ingredients	F0	F1	F2	F3	F4	F5	F6	F7
Wheat flour	30	28	28	14	28	13	16	8
Tapioca flour	7	7	7	4	6	3	4	2
Cornstarch	10	10	10	5	9	4	6	3
Tilapia fish flour	0	2	0	0	1	4	0	3
Sardine fish flour	0	0	2	0	3	0	2	3
Red rice flour	0	0	0	24	0	24	19	28
Egg yolk	29	29	29	29	29	29	29	29
Margarine	12	12	12	12	12	12	12	12
Sugar	12	12	12	12	12	12	12	12
Total ingredients	100	100	100	100	100	101	100	100

3.3. Biscuit Nutritional Content

The nutritional content studied are the macronutrient content, which includes energy, protein, fat, carbohydrate, water, ash, and crude fiber. For further detail, the nutritional content of eight type of biscuits can be seen in Table 2

Table 2. Nutritional content of eight biscuits

Nutrition Contents	F0	F1	F2	F3	F4	F5	F6	F7	Change
Energy (Kkal)	476,03	500,33	490,65	507,46	478,78	492,3	483,06	478,11	-1,1371x
Protein (g)	7,72	13,59	11,01	11,68	11,05	11,25	10,03	7,8	-0,2042x
Fat (g)	17,43	24,77	21,49	24,46	19,3	21,75	20,43	20,23	-0,0773x
Carbohydrate (g)	72,07	55,76	63,3	60,15	65,22	62,89	64,77	66,21	0,0939x
Water (g)	2,03	4,48	3,03	2,52	3,1	2,88	3,31	3,94	0,0910x
Ash (g)	0,75	1,4	1,17	1,19	1,33	1,23	1,46	1,82	0,0966x
Crude Fiber (g)	4,51	5,55	4,55	3,38	4,75	4,23	4,88	5,66	0,0607x

The nutritional content checked will be conducted on 8 biscuits produced which consist of 7 biscuits with substituted ingredients and 1 without substitution, which are: F1 (tilapia fish flour substitution 2%), F2 (sardine fish flour substitution 2%), F3 (red rice flour substitution 24%), F4 (tilapia fish flour substitution 1% and sardine fish flour substitution 3%), F5 (tilapia fish flour substitution 4% and red rice flour 24%), F6 (sardine fish flour substitution 2% and red rice flour 19%), and F7 (tilapia fish flour substitution 3%, sardine fish flour substitution 3%, and red rice flour 28%), and F0 is the non-substituted biscuit (control biscuit).

3.3.1. Energy

Statistically, the results for biscuit with substitution of tilapia fish flour, sardine fish flour, and red rice flour to the energy level of biscuit showed that energy decreased. The analysis result of the energy content of substitution of tilapia fish flour, sardine fish flour, and red rice flour can be seen in Figure 1.

Based on Figure 1, energy content analysis of biscuit with the substitution of tilapia fish flour and red rice flour is in the range of 476.03 Kcal to 507.46 Kcal. The tilapia fish flour, sardine fish flour, and red rice flour showed an energy decrease of 1.1371 times if the contents are greater. This is because the two substituents ingredients contribute less energy compared to the control biscuit. The research is in accordance with research conducted by Doportto, et al. which stated that the increase in energy content is influenced by its constituents, less fatty materials can reduce the energy content of the resulting biscuits [9], [10], and [11],

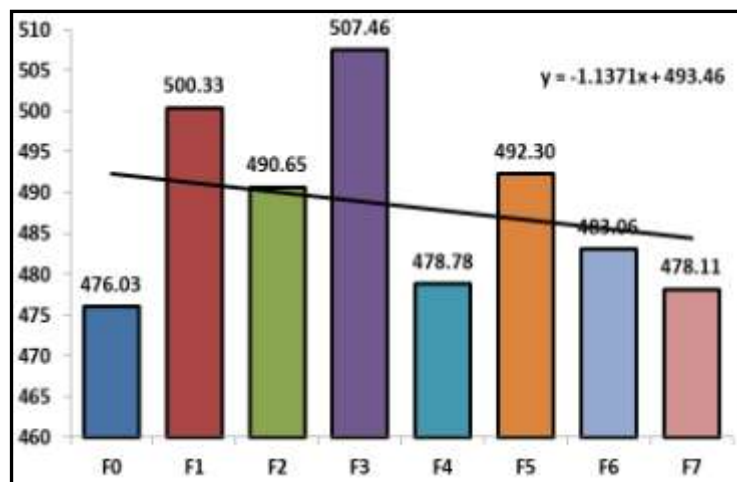


Figure 1. Energy content of biscuit with substitution of tilapia fish flour, sardine fish flour, and red rice flour

The energy level of biscuits per treatment is influenced by the carbohydrate, fat, and protein content of the main ingredients used. The content of the three nutrients of tilapia fish flour and red rice flour is lower than wheat flour. According to proximate results in previous studies, tilapia fish flour energy is 378.4 Kcal and red rice flour 331.6 [12]. Based on the SNI requirements of the general biscuit standard quality, the minimum protein value is at least 400 Kcal [8] and the energy content value in biscuits with substitution of tilapia fish flour, sardine fish flour, and red rice flour is 476.03-507.06 Kcal so that biscuits with the based of tilapia fish flour, sardine fish flour, and red rice flour have fulfilled the SNI requirement

The Ministry of Health of the Republic of Indonesia through The National Agency of Drug and Food Control of Republic of Indonesia or NADFC states that food can be regarded as an excellent source of energy if it contains at least 10% of the Recommended Dietary Allowance (RDA) per serving. If the Recommended Dietary Allowance (RDA) for the fourth-grade primary schoolers used is the Recommended Dietary Allowance (RDA) for children of 10-12 years, then 20% of 2,100 Kcal for boys and 2,000 Kcal for girls is the 10 grams of protein to be fulfilled from the dish. In average, the biscuits with the substitution of tilapia fish flour, sardine fish flour, and red rice flour fulfilled the requirements. Recommended Dietary Allowance (RDA) Percentage of the energy generated from 100 gram biscuits ranged in 22.7-25%. Therefore, base on the Recommended Dietary Allowance (RDA), the energy of biscuit is higher than what is set forth by the National Agency of Drug and Food Control of the Republic of Indonesia.

3.3.2. Protein

The results for biscuit substitution of tilapia fish flour, sardine fish flour, and red rice flour, and one control biscuit can be seen in Figure 2.

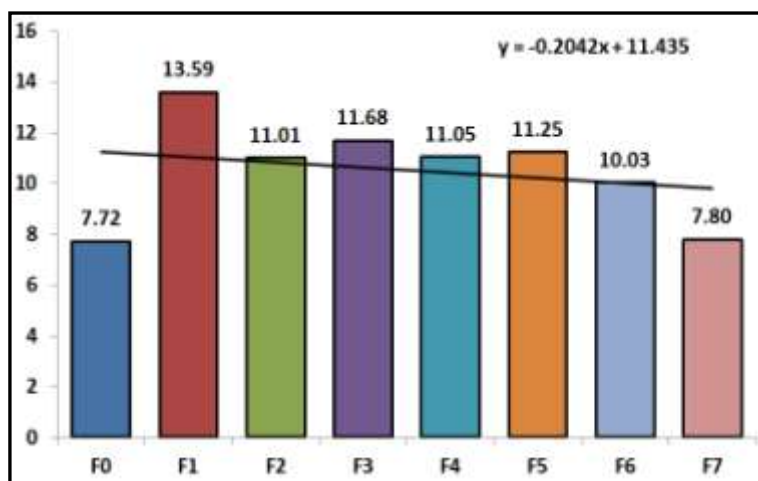


Figure 2. Protein content of biscuits with substitution of tilapia fish flour, sardine fish flour, and red rice flour

Based on Figure 2, the protein content analysis for biscuits with the substitution of tilapia fish flour, sardine fish flour, and red rice flour ranged in 7.72% to 13.59%. The greater the substitution of tilapia fish flour, sardine fish flour, and red rice flour, the lower the protein content of the biscuit, at almost 0.2042 times. This research is in line with research conducted by Listiana which states that the higher the substitution of swordfish flour in biscuits, the higher the protein content of 11% -19% [13], Meanwhile, Pang et al. conveyed the protein content change in Mi substitution of tuna fish is 11.20%-18.26% [14][15], An increase in protein was also delivered by Sari et al., that stated that biscuits substituted with cork fish flour increased the protein by 12% [16], The study of Widodo et al., also stated that biscuit protein increased by 14 % after substituted with blondo, cork fish flour, and red rice flour [11].

The increase of protein content in each treatment was influenced by the protein content of the base ingredients used in which the protein content of tilapia fish flour, sardine fish flour, and red rice flour were higher than wheat flour. According to the last study's proximate result, the protein in tilapia fish flour is 60.5% and the protein in red rice flour is 8.5% [12]. Based on the SNI requirements of the general biscuit standard quality, the minimum protein value is at least 5% [8][17][7][18] and the energy content value in biscuits with substitution of tilapia fish flour is 7.72%-13.59%, so the biscuit with substitution of sardine fish flour, and red rice flour have fulfilled the SNI requirement.

The Ministry of Health of the Republic of Indonesia through The National Agency of Drug and Food Control of Republic of Indonesia or NADFC states that food can be regarded as an excellent source of energy if it contains at least 20% of the Recommended Dietary Allowance (RDA) per serving. If the Recommended Dietary Allowance (RDA) for the fourth-grade primary schoolers used is the Recommended Dietary Allowance (RDA) for children of 10-12 years, then 20% of 50 gram protein is the 10 grams of protein to be fulfilled from the dish. The biscuits with the substitution of tilapia fish flour, sardine fish flour, and red rice flour overall have protein content of 10.03-13.59 grams, and only F0 and F7 are still below the minimum standard of 20% Recommended Dietary Allowances (DRA) per 100 gram serving. To meet the high protein criteria, the amount of biscuits consumed is 100 grams or 10 pieces per day for elementary school students.

The biscuit formula that substitutes tilapia fish flour, sardine fish flour, and red rice flour greatly aids the body in improving metabolism, so the body gets healthier because it fulfills its nutritional needs [19] [20], the nutritional contained in the biscuits may also avoid double disease in children and adolescents [21], reduce vitamin A and zinc deficiencies that ultimately improve the children's immune and improve vision [22] [23]. The content of quality ingredients in biscuits will affect the results and content of biscuits such as antioxidant content in fish flour and red rice flour. Antioxidants contained in the material will improve the nutrient content of the damaged material at the time of processing and become oxidant. Hence, ingredients that contain antioxidants are necessary [24][25].

3.3.3. Lemak Fat

The results for biscuit substitution of tilapia fish flour, sardine fish flour, and red rice flour, on the biscuits fat content, can be seen in Figure 3

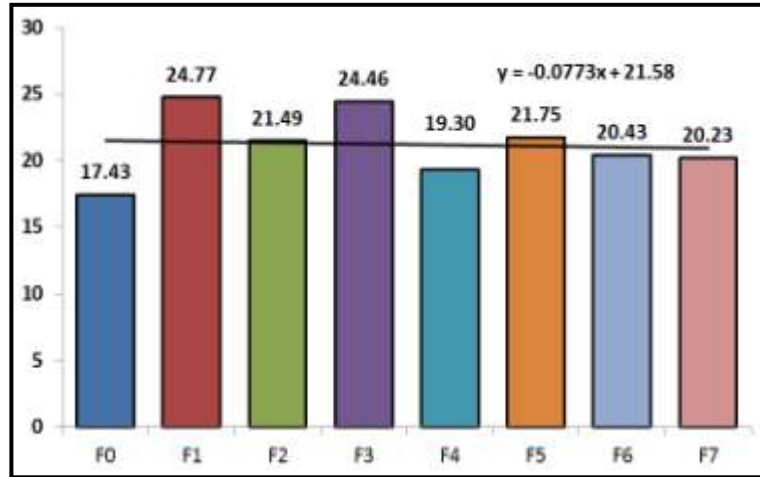


Figure 3. Fat content in biscuits with substitution of tilapia fish flour, sardine fish flour, and red rice flour

Based on Figure 3, the protein content analysis for biscuits with the substitution of tilapia fish flour, sardine fish flour, and red rice flour ranged in 17.43% to 24.77%. The biscuits with substitution have higher fat content than the control biscuit. The greater the substitution of tilapia fish flour, sardine fish flour, and red rice, the greater the fat content of biscuit produced, which decreased at 0.0773 times. This study is in line with research conducted by Izumi et al. who stated that the high substitution of high fat can increase the body fat content by 0.05 times compared with the low fat [26], Meanwhile, Niu et al. conveyed changes in fat content of complementary foods after the replacement of fish flour with soybean flour by 0.23 times compared to the previous [27], The increase in fat was also delivered by Sari et al., who stated that biscuits substituted with cork fish flour increased fat by 0.01 times [14], and the study of Widodo et al, also stated that biscuit fat increased by 4 times after being substituted with blondo, cork fish flour, and red rice flour [11][18].

The increase in fat content in each treatment is influenced by the fat content of the basic ingredients used in which the fat content of tilapia fish flour and red rice flour is lower compared with margarine and egg yolks. According to proximate results in previous studies, tilapia fish flour fat is 8.5% and red rice flour 1.3% [12]. Based on the SNI requirements of the general biscuit standard quality, the maximum fat value is 10-15% [7] and the fat content value in biscuits with substitution of tilapia fish flour is 17.43-24.77%. Hence, the biscuits with substitution of tilapia fish flour and red rice flour have fulfilled the SNI requirement.

3.3.4. Carbohydrate

Substitution effect of tilapia fish flour, sardine fish flour, and red rice flour to the biscuit carbohydrate content can be seen in Figure 4.

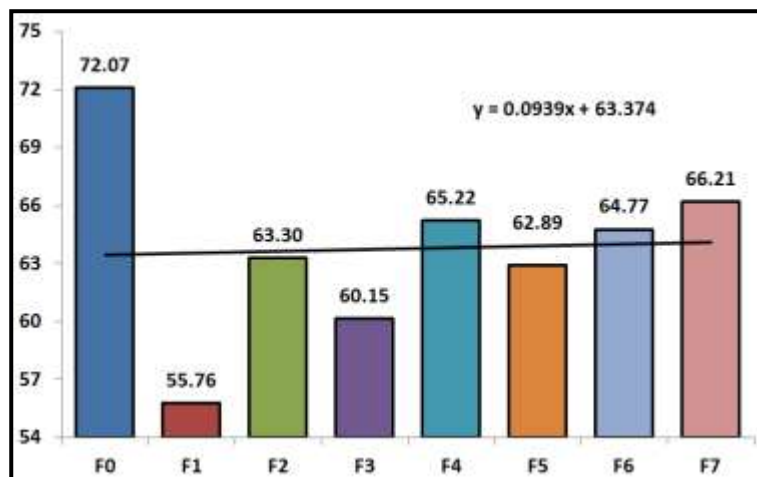


Figure 4. Carbohydrate content of biscuits with substitutions of tilapia fish flour, sardine fish flour, and red rice flour

Based on Figure 4, the carbohydrate content analysis for biscuits with the substitution of tilapia fish flour, sardine fish flour, and red rice flour ranged in 55.76% to 72.07%. The greater the substitution of tilapia fish flour and red rice flour, the greater the carbohydrate content of the biscuit, at 0.0939 times. This study is in line with a study conducted by Widodo et al., who stated that the higher the substitution material, the higher the increased carbohydrate content in biscuits is, by 0.65 times [11][25]. This is due to the carbohydrate content of each substitute material that is lower but the amount used is more, but still cannot exceed the wheat only biscuits.

Carbohydrate levels in each treatment increased slightly, as it was affected by low carbohydrate content in red rice flour and other substitutes. According to proximate results in previous studies, tilapia fish flour carbohydrate is 15.16% and red rice flour 71.42% [12].

3.3.5. Water

Substitution effect of tilapia fish flour, sardine fish flour, and red rice flour to the biscuit water content can be seen in Figure 5.

Based on Figure 5, the water content analysis for biscuits with the substitution of tilapia fish flour, sardine fish flour, and red rice flour ranged in 55.76% to 72.07%. The greater the substitution of tilapia fish flour and red rice flour, the greater the water content of the biscuit, at 0.091 times. The study is in line with research conducted by Widodo et al., who stated that higher the substitution ingredients, the higher the water content in biscuits at 0.65 times [11]. This is due to the water content of each substitute material that is higher but the amount used is still more, but still cannot exceed the wheat only biscuits.

The water content in each treatment increased was slightly influenced by low water content in tilapia fish flour, sardine fish flour, and red rice flour. According to proximate results in previous studies, tilapia fish flour water content is 2.26%, sardine fish flour water content is 2.35%, and red rice flour 16.54% [12].

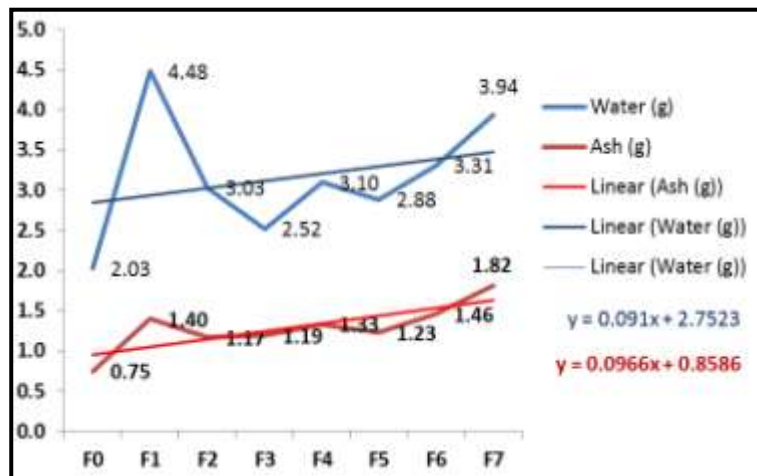


Figure 4. Water and ash content of substitutions of tilapia fish flour, sardine fish flour, and red rice flour

The result of water content analysis on the substitution of tilapia fish flour, sardine fish flour, and red rice flour were water content of less than 5%. The lowest water content was found with the addition of red rice flour 2.52% while the highest water content was obtained in the treatment of 4.48% tilapia fish flour addition. This is in accordance with the standard, in which the water content in biscuits is below 5% (SNI, 2011). Tilapia fish flour, sardine fish flour, and red rice flour were found to have increased the water content of biscuits by 0.091 times. Substitution of tilapia fish flour, sardine fish flour, and red rice flour on each treatment has fulfilled the SNI standard. The biscuit making process used high temperatures and baking. The temperature used is 150°C. The temperature can reduce the water content in the ingredients, so the water content in the biscuit is low. The low water content in the biscuit affects the crispness, which is a mandatory attribute on biscuits. According to Ayuning, low water content on biscuits will affect the texture and will be preferred by consumers [28]

Water content will be more difficult to lose when there are inhibitory ingredients in the product. These inhibiting agents will affect the results of the water content test. Inhibitors of water analysis are mostly carbohydrates. Fiber is one type of carbohydrate found in red rice. The content of fiber in biscuits with substitution is 3.38-5.66%. Inhibitors in water content analysis are glucose, maltose, lactose, and other hydrate compounds [17]. The result of water content analysis on the substitution of tilapia fish flour, sardine fish flour, and red rice flour were water content of less than 5%. The lowest water content was found with the addition of red rice flour 2.52% while the highest water content was obtained in the treatment of 4.48% tilapia fish flour addition. This is in accordance with the standard, in which the water content in

biscuits is below 5% (SNI, 2011) Tilapia fish flour, sardine fish flour, and added red rice flour did not have a significant effect on the water content of the biscuits. Increased water content in biscuits is influenced by the amount of substitution of tilapia fish flour, sardine fish flour, and red rice flour. However, tilapia fish flour, sardine fish flour, and red rice flour are suitable for biscuit making because they still fulfill the SNI standards of water content in biscuits. The water content of less than 5% can prolong the shelf life and avoid the microbes that can damage the biscuit [11] [12].

Water content will be more difficult to lose when there are inhibitory ingredients in the product. These inhibiting agents will affect the results of the water content test. Water content analysis with a 40% concentration of sweet potatoes has the highest water content. This is influenced by the inhibitor. Inhibitors of water analysis are mostly carbohydrates. Fiber is one type of carbohydrate found in sweet potato. The content of fiber in sweet potato biscuit with a substitution of 40% is 8.88% and considered as high. Inhibitors in water content analysis are glucose, maltose, lactose, and other hydrate compounds. The water content will affect the texture. Water content increase will affect the crispness in biscuits. The higher the water content, the less crunchy the biscuit will be. Treatment of 40% sweet potato addition has high moisture content. Biscuit's crunchiness level is low crispness. This proved that moisture content will affect the texture. The softness of a food product will affect the texture affected by the high water content. [17].

3.3.6. Abu Ash

Substitution effect of tilapia fish flour, sardine fish flour, and red rice flour to the biscuit ash content can be seen in Figure 5

Based on Figure 5, the ash content analysis for biscuits with substitution of tilapia fish flour, sardine fish flour, and red rice flour ranged in 55.76% to 72.07%. The greater the substitution of tilapia fish flour and red rice flour, the greater the ash content of the biscuit, at 0.0966 times. The study is in line with research conducted by Widodo et al., who stated that higher the substitution ingredients, the higher the water content in biscuits at 0.072 times [11]. This is due to the ash content of each substitute material that is higher but the amount used is still more, but still cannot exceed the ash standard in biscuits, which is 2%.

The level of ash in each treatment increased slightly influenced by tilapia fish flour, sardine fish flour, and red rice flour. According to proximate results in previous studies, tilapia fish flour ash content is 13.17%, sardine fish flour ash content is 13.63%, and red rice flour is 0.81% [12]. Ash content is a mixture of inorganic components or minerals contained in a foodstuff. Foodstuffs comprise of 96% of inorganic and water materials, and the remainder is mineral elements. Elements are also known as organic substances or ash content. The ash content may indicate the total minerals in a foodstuff. The organic materials in the combustion process will burn but the inorganic components will not. Hence, it is called ash content. Determination of total ash content may be used for various purposes, among others, to determine whether or not a processing, to know the type of material used, and as a determinant of the parameters of the nutritional value of a foodstuff. The ash content can also be used to estimate the content and authenticity of the ingredients used [29][30][31].

Ash is an inorganic residue of organic compounds combustion. In addition to ash, there is another component in foodstuff, namely mineral. The ash level in foodstuff affects the nature of the foodstuff. Ash content is a measure of the total amount of minerals contained in the foodstuff. This demonstrates that the determination of water content greatly affects the mineral content determination. Mineral content is a measure of the number of certain inorganic components present in foodstuffs, such as Ca, Na, K, and Cl. Determination of total ash content may be used for various purposes, among others, to determine whether or not a processing, to know the type of material used, and as a determinant of the parameters of the nutritional value of a foodstuff [29][30][31].

4. CONCLUSION

The macronutrients content in the nutritious biscuits with the substitution of tilapia fish flour, sardine fish flour, and red rice flour include F0 (without substitution) of nutrient content 476,03 kcal energy, 7.72 g protein, 17.43 g fat, 72.07 g carbohydrate, 2.03 g water, and 0.75 g ash. F1 (substitution of tilapia fish flour 2%) nutrient content is 500.33 Kcal energy, 13.59 g protein, 24.77 g fat, 55.76 g carbohydrates, 4.48 g water, and 1.4 g ash. F2 (substitution of sardine fish flour 2%) nutrient content is 490.65 Kcal energy, 11.01 g protein, 21.49 g fat, 63.3 g carbohydrates, 3.03 g water, and 1.17 g ash. F3 (substitution of red rice flour 29%) nutrient content is 507.46 Kcal energy, 11.68 g protein, 24.46 g fat, 60.15 g carbohydrates, 2.52 g water, and 1.19 g ash. F4 (substitution of tilapia fish flour 1% and sardine fish flour 3%) nutrient content is 478.78 Kcal energy, 11.05 g protein, 19.3 g fat, 65.22 g carbohydrates, 3.1 g water, and 1.33 g ash. F5 (substitution of tilapia fish flour 4% and red rice flour 24%) nutrient content is 492.3 Kcal energy, 11.25 g protein, 21.75 g fat, 62.89 g carbohydrates, 2.88 g water, and 1.23 g ash. F6 (substitution of sardine fish flour 2% and red rice flour 19.3%) nutrient content is 483.06 Kcal energy, 10.03 g protein, 20.43 g fat, 64.77 g carbohydrates, 3.31 g water, and 1.46 g ash. F7 (substitution of tilapia fish flour 3%, sardine fish flour 3%, and red rice flour 28%) nutrient content is 478.11 Kcal energy, 7.8 g protein, 20.23 g fat, 66.21 g carbohydrates, 3.94 g water, and 1.82 g ash.

5. ACKNOWLEDGEMENT

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6. REFERENCES

- [1] R. H. Steckel, *Malnutrition Global economic losses attributable to malnutrition 1900- 2000 and projections to 2050*. Ohio State University: Copenhagen Consensus on Human Challenges, 2013.
- [2] C. G. Victora *et al.*, “Maternal and child undernutrition: consequences for adult health and human capital,” *Lancet*, vol. 371, no. 9609, pp. 340–357, 2008.
- [3] M. Islam and M. Taneya, “Physicochemical and Functional Properties of Brown Rice (*Oryza sativa*) and Wheat (*Triticum aestivum*) Flour and Quality of Composite Biscuit Made Thereof,” *A Sci. J. Krishi Found. Agric.*, vol. 10, no. November, pp. 20–28, 2012.
- [4] S. A. Shaji and C. K. Hindumathy, “Chemical composition and amino acid profile of *Sardinella longiceps* collected from Western coastal areas of Kerala , India,” *J. Biol. Earth Sci.*, vol. 3, no. 1, pp. B129–B134, 2013.
- [5] B. Wosniak *et al.*, “Effect of diets containing different types of sardine waste (*Sardinella sp .*) protein hydrolysate on the performance and intestinal morphometry of silver catfish juveniles (*Rhamdia quelen*),” *Lat. Am. J. Aquat. Res.*, vol. 44, no. 5, pp. 957–966, 2016.
- [6] S. Widodo, H. Riyadi, I. Tanziha, and M. Astawan, “Improving nutritional status of children under five year by the intervention of blondo, snakehead fish (*Channa striata*), and brown rice (*Oryza nivara*) based biscuit,” *J. Gizi Pangan*, vol. 10, no. 2, pp. 85–92, 2015.
- [7] E. W. Riptanti and A. Qonita, “The Development of Sustainable Community Food Barn in Wonogiri Regency , Central Java , Indonesia,” *Asian J. Appl. Sci.*, vol. 5, no. 2, pp. 274–279, 2017.
- [8] B. S. N. Indonesia, *SNI BISKUIT*. Jakarta: BSN Indonesia, 2011.
- [9] M. C. Doportto, F. Sacco, S. Z. Viña, and M. A. García, “Quality and Technological Properties of Gluten-Free Biscuits Made with *Pachyrhizus ahipa* Flour as a Novel Ingredient,” pp. 70–83, 2017.
- [10] A. K. M. M. Hossain, M. A. Brennan, S. L. Mason, X. Guo, X. A. Zeng, and C. S. Brennan, “The Effect of Astaxanthin-Rich Microalgae ‘ *Haematococcus pluvialis* ’ and Wholemeal Flours Incorporation in Improving the Physical and Functional Properties of Cookies,” 2017.
- [11] S. Widodo, H. Riyadi, I. Tanziha, and M. Astawan, “Acceptance Test of Blondo , Snakehead Fish Flour and Brown Rice Flour based Biscuit Formulation,” *Int. J. Sci. Basic Appl. Res.*, vol. 4531, pp. 264–276, 2015.
- [12] S. Widodo and S. Sirajudin, “Effect of Drying Time on Quality of Mozambique Tilapia Fish (*Oreochromis Mossambicus*) and Round *Sardinella* (*Sardinella Aurita*) Flour,” in *International Conference ADRI - 5 (“Scientific Publications toward Global Competitive Higher Education”)*, 2017, pp. 157–163.
- [13] L. Listiana, “Pengaruh Substitusi Tepung Ikan Tongkol terhadap Kadar Protein, Kekerasan dan Daya Terima Biskuit,” *J. UMS*, vol. 2, pp. 1–13, 2016.
- [14] C. J. Pang, E. Noerhartati, and F. S. Rejeki, “Optimasi Proses Pengolahan Mi Ikan Tongkol (*Euthynnus Affinis*),” *J. REKA Agroindustri*, vol. I, no. 1, pp. 1–7, 2013.
- [15] T. Agung *et al.*, “Genetic Studies on Grain Protein Content and Some Agronomic Characters of Rice by Halfdiallel Crossing System,” *Asian J. Appl. Sci.*, vol. 5, no. 2, pp. 461–466, 2017.
- [16] D. K. Sari, S. A. Marliyati, L. Kustiyah, and A. Khomsan, “Role of Biscuits Enriched with Albumin Protein from Snakehead Fish , Zinc and Iron on Immune Response of under Five Children,” vol. 13, no. 1, pp. 28–32, 2014.
- [17] N. Andarwulan, F. Kusnandar, and D. Herawati, *Analisis Pangan*. Jakarta: PT. Dian Rakyat, 2011.
- [18] S. M. A. Villota, A. M. Tuates, and O. A. Capariño, “Cooking Qualilites and Nutritional Contents of Parboiled Milled Rice,” *Asian J. Appl. Sci.*, vol. 4, no. 5, pp. 1172–1178, 2016.
- [19] J. A. Ogbonnaya, A. Ketiku, A. Sanusi, and T. Adepoju, “Nutrient Density , Acceptability , and Bioavailability of Iron and Zinc in Fish Flour – and Liver Flour – Enriched Complementary Foods,” *Advand Nutr.*, vol. 7, no. 21A, pp. 7–8, 2016.
- [20] K. Hanhineva *et al.*, “Nontargeted Metabolite Profiling Discriminates Diet-Specific Biomarkers for Consumption of Whole Grains , Fatty Fish , and Bilberries in a Randomized Controlled Trial,” no. C, pp. 1–11, 2015.
- [21] E. Atalah, H. Amigo, and P. Bustos, “Does Chile ’ s nutritional situation constitute a double burden ? 1 – 4,” *Am. J. Clin. Nutr.*, vol. 100, no. 1, p. 1623S–1627S, 2014.
- [22] S. T. Children *et al.*, “Triple-Fortified Rice Containing Vitamin A Reduced Marginal Vitamin A Deficiency and Increased Vitamin A Liver Stores in,” no. 4, pp. 519–524, 2014.
- [23] E. Chomba *et al.*, “Zinc Absorption from Biofortified Maize Meets the Requirements of Young Rural Zambian,” 2015.
- [24] A. Korus, D. Gumul, M. Krystyjan, and L. Juszcak, “Evaluation of the quality , nutritional value and antioxidant activity of gluten-free biscuits made from corn-acorn flour or corn-hemp flour composites,” no. 123456789, 2017.

- [25] K. E. K. V, M. Alassane, B. K. G. M, and K. K. Gustave, “Nutritional Performances of Diet Made with Soya / Maize and ‘ Pistachio ’ / Rice in the Rehabilitation of Underfed Rats,” *Asian J. Appl. Sci.*, vol. 4, no. 5, pp. 1072–1078, 2016.
- [26] M. Izumi, E. Manabe, S. Uematsu, A. Watanabe, and T. Moritani, “Changes in autonomic nervous system activity , body weight , and percentage fat mass in the first year postpartum and factors regulating the return to pre- pregnancy weight,” *J. Physiol. Anthropol.*, pp. 1–7, 2016.
- [27] J. Niu *et al.*, “Effect of Replacing Fish Meal with Soybean Meal and of DL Methionen or Lisiene Supplementation in Pelleted Diets on Growth and Nutrient Utilization of Juvenile Golden Pompane,” *Aquac. Nutr.*, no. 2013, pp. 1–9, 2015.
- [28] A. N. Jagat, Y. B. Pramono, and Nurwantoro, “Fiber Enrichment on Biscuit by Substitution of Yellowish Sweet Potato Flour (*Ipomea batatas L.*),” *J. Apl. Teknol. Pangan*, vol. 6, no. 2, pp. 4–7, 2017.
- [29] B. Sezer, G. Bilge, T. Sanal, H. Koxsel, and I. H. Boyaci, “A novel method for ash analysis in wheat milling fractions by using laser-induced breakdown spectroscopy,” *J. Cereal Sci.*, 2017.
- [30] C. Huang, X. Wu, Y. Huang, C. Lai, X. Li, and Q. Yong, “Prewashing enhances the liquid hot water pretreatment efficiency of waste wheat straw with high free ash content,” *Bioresour. Technol.*, 2016.
- [31] H. Juan, “An integrated process to produce bio-ethanol and xylooligosaccharides rich in xylobiose and xylotriose from high ash content ...,” *Bioresour. Technol.*, vol. 241, no. October, pp. 228–235, 2017.