Substitution of patin *Pangasius pangasius* flour in making sticks as an alternative of food high protein and source of calcium for autism patients

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Abstract. The wrong food choice is one of the causes of the increasing prevalence of autism in Indonesia. Casein Free and Gluten Free (CFGF) therapy can be done to minimize this problem. The application of CFGF therapy can reduce the nutritional value of protein, and calcium in people with autism. Patin flour is a diversified processed fishery product that has a higher nutritional value than wheat flour. The purpose of this study was to determine the effect of substitution of patin flour on the making of sticks as an alternative to high protein food, and a source of calcium for autism sufferers. This study used a completely randomized design (CRD) method with 4 treatments, namely the percentage of patin flour (0, 22, 27, and 32% w/w). The results of this study indicated that the substitution of patin flour had a significant effect (p<0.05) on the levels of protein, and calcium produced. 32% substitution of patin flour is the treatment with the highest protein, and calcium. Based on the provisions of the BPOM, it can be used as high protein food, and a source of calcium in meeting the nutritional adequacy rate every day.

1. Introduction
Autism or *Autistic Spectrum Disorder* (ASD) is a developmental disorder in a person which includes social interaction disorders, communication disorders, and behavior disorders [1]. Based on data from the World Health Organization (WHO), it shows that the prevalence of autism in Indonesia in 2016 has increased rapidly compared to the previous 10 years, namely from 1 per 1000 population to 8 per 1000 population. This figure even exceeds the world average of 6 per 1000 population [1].

The increasing prevalence of autism sufferers can be caused by several factors, one of which is the wrong selection of foods. Autism sufferers tend to have allergies to foods containing gluten, and casein which will affect their behavior disorders [2]. Based on research by Megawati [3] states that 72.3% of autism sufferers still consume foods that are sourced from gluten and casein.

Efforts that can be made to minimize behavioral disorders due to inappropriate feeding are *Casein
Free and Gluten Free (CFGF) therapy, namely foods that are free of gluten and casein [3]. However, food choices that support CCFG therapy tend to reduce the nutritional value of protein and calcium in autism sufferers, so it is necessary to increase the nutritional value of protein, and calcium to meet the daily nutritional needs of autism sufferers.

Cookies, sticks, and the like are the most preferred snacks for autism sufferers due to their affordable prices, easy access, and varying flavors [3]. But on the other hand, cookies, sticks, and the like are foods made from wheat flour, which is a source of gluten, so a reformulation is needed by substituting wheat flour into patin meat flour.

Patin meat flour is one of the diversified processed fisheries products that have a higher nutritional value than vegetable flour. Patin meat has a higher protein, and calcium content when compared to wheat flour. Protein in patin meat flour is 67.76g / 100g of ingredients, while in wheat flour is 10.00g / 100g of ingredients [4]. Calcium of patin meat flour is 435mg/100g of ingredients, while in wheat flour it is 15mg /100g of ingredients [5]. In addition, fish meat has shorter protein fibers so that it is easier to digest than beef or chicken protein so that the products are widely used by people who have digestive difficulties, especially those with autism. Therefore, in this study, the substitution of patin meat flour was carried out to determine the effect of protein, and calcium levels on the making of sticks so that they can be used as an alternative food for autism sufferers.

2. Materials and methods

The research was conducted in March - June 2020. The characterization of patin meat flour (proximate analysis) was carried out at the Chemical Analysis Laboratory of the Faculty of Fisheries and Marine, Universitas Airlangga, and analysis of calcium content of patin meat was carried out in the Laboratory Nutrition, Faculty of Public Health, Universitas Airlangga.

The chemical analysis of the sticks was carried out at the Animal Feed Laboratory of the Faculty of Veterinary, Universitas Airlangga. The stick sensory test was carried out in a private home.

The equipment used in this study is divided into three parts, namely: first, the equipment used in the making of patin meat flour including knives, cutting boards, pans, stopwatches, blenders, plates, digital scales, basins, and filters. Second, the equipment used in making sticks includes digital scales, pans, stoves, knives, trays, spoons, and basins. Third, the equipments for chemical analysis and sensory tests.

The materials used in this study are patin (Pangasius pangasius), tapioca flour, eggs, garlic, shallots, butter, sugar, and salt. The supporting materials used in the manufacture of sticks are cold water and cooking oil.

2.1 Making patin meat flour

The material used in this stage procedure is patin (Pangasius pangasius) which has been weighed, and continued with the weeding process. Furthermore, a second weighing is carried out, followed by steaming for 10 minutes. After that, the meat is separated from the bone, and skin that is still attached. The next step is weighing the fish meat that has undergone the steaming process. Weighing is done to calculate the yield. Then the meat is soaked in lime juice for 30 minutes with the aim of eliminating the fishy smell of fish. Then after that the pressing is carried out, and followed by the oven process with a temperature of 50°C for ± 16 hours, after it is dry, smoothing it using a blender, then filtering it using a sieve so that a fine fish flour is produced and the final weighing is carried out [5].

2.2 Characteristic of patin meat flour

The resulting fish flour was subjected to a proximate analysis which included water analysis, protein analysis, fat analysis, ash analysis, fiber analysis, carbohydrate analysis, and calcium analysis.

2.3 Making sticks

Based on the research of [6] making sticks begins with making dough with a formulation of 14% wheat flour, 54% tapioca flour, 32% other ingredients, and 0% fish flour. Then in this study, the
formulation modification was carried out based on the best results from the research of Sari et al. [6] namely variations in the concentration of patin meat flour 0%, 22%, 27% and 32%. Add other ingredients, namely eggs, onion, garlic, salt, sugar, water, and margarine. Followed by making dough and stirring until homogeneous. Then the formation of thin sheets of dough and printing of sticks with a thickness of ± 3 mm and a length of 8-10 cm. Cook the sticks by frying the dough with a volume of 1 liter of oil until the sticks are brownish yellow (± 3 minutes), and the last step is cooling and draining the sticks for 5 minutes at room temperature [6].

2.4. Testing of sticks with patin meat flour substitution
Tests carried out for the results of sticks with patin meat flour substitution include yield calculations, chemical testing including water analysis, ash analysis, protein analysis using the Kjeldahl method, fat analysis using the Soxhlet method, carbohydrate content using the by different method, calcium content using the the AAS (Atomic Absorption Spectrophotometer) method and sensory test which refers to SNI 01-2346-2006.

2.5. Data Analysis
Data analysis includes chemical analysis was carried out statistically using the Statistical Product Service Solution (SPSS) program with the One Way Anova test to determine whether there was a difference between each treatment and if the treatment had a significant effect, then continued using the Duncan test [7].

Non-parametric data, namely sensory test results were analyzed using the Kruskal-Wallis test. The Kruskal-Wallis test was used to rank each observation parameter and if the treatment had a significant effect, it was continued using the Mann - Whitney test.

3 Result and Discussion
3.1. Characteristic of patin meat flour
The patin meat flour produced in this study has a yellowish brown color and has a smooth texture. Patin meat flour can be seen in Figure 1.

![Figure 1. Patin meat flour (Pangasius pangasius)](image)

3.2. Yield of patin meat and flour
The result yield of patin meat and flour can be seen in Table 1.

| Yield          | Result (%) |
|----------------|------------|
| Yield of meat  | 29.44      |
| Yield of flour | 6.25       |

3.3. Proximate test of patin meat flour
The result proximate test of patin meat flour can be seen in Table 2.
3.4. Sensory test

Stick sensory test with 4 parameters, namely appearance, smell, taste, and texture. In the sensory test for this stick, it uses the criteria according to the SNI 01-2346-2006 standard. The average data of the sensory stick test results can be seen in Table 3. The pictures of the sticks in each treatment can be seen in Figure 2.

| Component   | T1 ± DS     | T2 ± DS     | T3 ± DS     | T4 ± DS     |
|-------------|-------------|-------------|-------------|-------------|
| Appearance  | 8.37 ± 0.55 | 7.10 ± 0.75 | 6.83 ± 0.65 | 6.97 ± 0.85 |
| Smell       | 5.70 ± 0.66 | 6.20 ± 0.66 | 7.43 ± 0.56 | 8.36 ± 0.71 |
| Taste       | 7.00 ± 0.74 | 7.50 ± 0.57 | 7.93 ± 0.36 | 8.56 ± 0.50 |
| Texture     | 6.93 ± 1.61 | 4.00 ± 2.15 | 3.93 ± 1.01 | 2.87 ± 1.27 |

Note: T1 (0% patin meat flour), T2 (22% patin meat flour), T3 (27% patin meat flour), T4 (32% patin meat flour). The notation indicated by different superscript letters in the same column indicates a significant difference between treatments (p<0.05).

Figure 2. Stick in each treatment. P1 (0% patin meat flour), P2 (22% patin meat flour), P3 (27% patin meat flour), P4 (32% patin meat flour). The notation indicated by different superscript letters on the same line indicated a significant difference between treatments (p<0.05).

3.5. Protein Analysis

Data from the protein analysis on sticks with different percentages of patin meat flour substitution can be seen in Table 4. The percentage contribution of protein analysis in each sticks treatment in fulfilling the nutritional adequacy ratio (RDA) for each day can be seen in Table 5.
The percentage contribution of calcium concerning Recommended Nutritional Adequacy Rates for Indonesian People. Note: *.

Data from the Table 6. The notation indicated by different superscript letters in the same column indicated a significant difference between treatments (T<0.05).

Table 6. Result of calcium analysis

| Treatments | Average ± DS (%) | Percentage RDA of 100 g sticks |
|------------|------------------|-------------------------------|
| T1         | 0.12±0.001       | T1 (0%)                        |
| T2         | 0.14±0.002       | T2 (22%)                       |
| T3         | 0.15±0.004       | T3 (27%)                       |
| T4         | 0.18±0.002       | T4 (32%)                       |

Note: Notation indicated by different superscript letters in the same column indicates a significant difference between treatments (p<0.05).

3.6. Calcium Analysis

Data from the calcium analysis on sticks with different percentages of patin meat flour substitution can be seen in Table 6. The percentage contribution of calcium analysis in each sticks treatment in fulfilling the Nutritional Adequacy Rate (RDA) for each day can be seen in Table 7.

Table 7. Contribution of sticks calcium analysis in the fulfillment of Nutritional Adequacy Score

| Age Group (years) | *RDA Calcium (mg) | Percentage RDA of 100 g sticks |
|-------------------|-------------------|-------------------------------|
| 1 – 9             | 1,000             | T1 (0%)                        |
| 10 – 18           | 1,200             | T2 (22%)                       |
| 19 – 29           | 1,100             | T3 (27%)                       |
| 30 – 80+          | 1,000             | T4 (32%)                       |

Note: * RDA Based on Regulation of the Minister of Health of the Republic of Indonesia Number 28 of 2019 concerning Recommended Nutritional Adequacy Rates for Indonesian People.
3.7. Discussion

The brownish color of the patin meat flour is due to the drying process. Drying will produce dark colored material due to the effect of a browning reaction or maillard reaction, which is the reaction between carbohydrates and proteins, especially reducing sugars with the primary amino acid groups present in the material so that it will produce a brown material called melanoidin [8].

The fish yield in this study shows that the yield value of patin flour has decreased from the yield value of patin meat. The decrease in yield from patin meat was due to a reduction in weight from whole fish to mashed meat, namely the separation of the head, stomach contents and fins which were not used in the study so that it was also followed by a decrease in the yield of fish flour caused by the process of making fish flour, such as: steaming, grinding, drying, and sieving.

Proximate analysis of patin meat flour showed the highest percentage results, namely protein content, this is because fish meat is mostly composed of myofibrilar protein, which is 60-75% protein in muscle used for fish movement [9]. Fat analysis in a flour product derived from fish raw materials is expected to be of low value, because the lower the fat content of a product, the higher the shelf life of the product [9]. The water content of patin is related to the shelf life of fish flour. The higher the water content of a material, the higher the possibility of the material being damaged [10]. The ash content of fish flour is influenced by the raw materials used, the ash content of the flour will be high if the raw materials come from the fish head and bones. Carbohydrates are one of the components in food ingredients as the main energy needed by the body, this is because carbohydrates play a role in providing glucose in body cells which will then be processed into energy [11].

Based on the BPOM [12] states that food can be explained to be high in protein if it contains at least 30% of the Nutritional Adequacy Rate (RDA). Based on these provisions, the percentage results that have been fulfilled for all age groups, namely at T4 (substitution of patin meat flour 32%) have contributed between 30.01-78.44% in daily adequacy per 100g of sticks so that they can be used as formulations for food with the high protein.

The high protein content percentage will be more sufficient for the nutritional needs of autism sufferers, considering that in the body of autism sufferers the protein content is low due to the Casein Free Gluten Free (CFGF) diet. Enough protein for autism sufferers can build muscle structure, make enzymes that control every chemical reaction in the body, make variations in brain neurotransmitters and hormones that are needed [13].

Based on BPOM [14], food can be said to be a source of calcium if it contains at least 15% of the Nutritional Adequacy Rate (RDA) and is suggested to be high in calcium if it contains at least 30% of the Nutritional Adequacy Rate (RDA). Based on these provisions, the percentage results that have met the minimum limit of calcium sources for all age groups, namely at T4 (substitution of patin flour 32%) are around 15-18% so that they can be used as a formulation for calcium source foods.

High calcium content is needed for the prevention of osteoporosis which is characterized by decreased bone strength, and increased brittleness caused by low bone density [15]. One of the groups that are at risk of experiencing calcium deficiency, and tend to develop osteoporosis is autism sufferers due to restrictions on foods containing casein protein which is abundant in milk and its processed foods, which are food sources of vitamin D, and calcium [16].

According to Meilgaard et al., [17] appearance is one of the attributes of product appearance that often determines the level of consumer acceptance of the product as a whole. Based on the measurement of the degree of whiteness of the flour, patin flour has a lower whiteness value than wheat flour, the meaning that the more fish meat is added, the darker the resulting sticks will affect the color on the appearance of the sticks.

According to Ratnawati [18] an evaporating smell, and taste is an attribute of a product that is received by the olfactory cells in the nose, and transmitted to the brain in the form of electrical impulses. Based on the organoleptic results, it shows that the higher the substitution of patin flour, it will cause a strong fish odor. Same thing with the smell, if the higher the substitution of patin flour, it will cause a strong fish taste.

Texture is complex, and is related to the structure of the material which consists of three elements,
namely mechanical (hardness, elasticity), geometric (sandy, weak), and mouthfeel (oily, watery) [10]. The results shown in this study that the higher the substitution of patin meat flour cause the lower the texture value.

4 Conclusion

The conclusion of this study is that the substitution of patin meat flour has an effect on the protein content, and the calcium level to increase. Based on this research, the results show that substitution of patin meat flour 32% is the treatment with the highest protein, and calcium content compared to other formulations and based on the provisions of the BPOM that formulation of substitution of patin meat flour 32% can be used as the high protein food, and a source of calcium in the nutritional adequacy rate for every day.

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