Jurnal Ilmiah Farmasi (Scientific Journal of Pharmacy) 21(1) January-May 2025, 58-65
ISSN: 1693-8666
available at http://journal.uii.ac.id/index.php/JIF

Analysis of protein, fat, and iron in catfish and green spinach biscuits

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Abstract

Background: Stunting in Indonesia remains a significant health issue that cannot be overlooked. Stunting can be addressed through the consumption of foods derived from both animal and plant sources, which are high in protein and rich in essential amino acids beneficial for preventing stunting. The provision of biscuits fortified with catfish and spinach flour is anticipated to assist the government in reducing the prevalence of stunting.

Objective: The aim of this study was to develop a functional food product formula utilizing catfish and spinach flour, while also analyzing the nutritional content of the resultant biscuits.

Method: This research employed three biscuit formulas with varying ratios of catfish and spinach flour specifically F1 (15 g : 45 g), F2 (30 g : 30 g), and F3 (45 g : 15 g). Chemical analyses conducted on the biscuits included tests for moisture content and ash content using the gravimetric method, protein analysis utilizing the Kjeldahl method, fat analysis via the Soxhlet extraction method, and carbohydrate analysis employing the titrimetric method.

Results: Based on the tests of the three biscuit formulas, it was found that the biscuits with formula F3 meet the minimum requirements outlined by the SNI, except for ash content. The protein content was 11.03%, the fat content was 31.073%, the water content was 2.26%, and the ash content was 2.98%. The protein content in formula F3 was higher than that in formulas F1 and F2 and has a high enough iron content at 5.025 mg/100 g.

Conclusion: It can be concluded that biscuits with F3 formula meet the nutritional values required by SNI regarding protein, fat, and water content. This formula has an opportunity for further development to enhance the carbohydrate, iron, and ash content of the biscuit to comply with SNI standards.

Keywords: Catfish biscuit, food fortification, processed food, spinach biscuit, stunting

1. Introduction

Stunting remains a significant nutritional challenge for the Indonesian government. Stunting is a growth disorder that occurs during the early stages of a child's life and can result in permanent damage (UNICEF/WHO/World Bank Group, 2021). Furthermore, addressing stunting is one of the objectives of the Sustainable Development Goals (SDGs), specifically related to Goal 2: Zero Hunger, which aims to reduce stunting by 40% by 2025 (Kemenkes RI, 2020).

Stunting is caused by inadequate nutrient intake, particularly protein and iron, as well as limited knowledge among parents regarding nutrition and the preparation of nutritious foods. These factors adversely affect the nutritional intake of children from conception to birth (Handarini & Madyowati, 2021; Rahmawati *et al*., 2021). Stunting requires special attention and must be prevented, as it is associated with decreased intellectual abilities and productivity, as well as an increased risk of degenerative diseases in adulthood. The growth of brain cells, which occurs rapidly, typically ceases or reaches its peak by the age of 4-5 years. Consequently, optimal brain development can only be achieved if the child attains a good nutritional status (Mohamad *et al*., 2022).

Efforts to improve the nutritional status of toddlers can be made by developing supplementary feeding formulas to enhance their nutritional value. One form of supplementary food is biscuit, a dry food product that is processed by baking dough using wheat flour, fat, and leavening agents. Biscuit can be formulated by incorporating other permitted food items, such as local ingredients (Wijinindnyah *et al*., 2022). Utilizing local food sources can serve as an alternative to address limited access to food for families and help mitigate food insecurity and malnutrition (Sutyawan *et al*., 2022). Examples of local food ingredients from Kalimantan that can be processed into biscuits include catfish and green spinach. These ingredients are commonly consumed by the local population, readily available, and relatively affordable.

Food plays an important role in the growth of toddlers. Addressing malnutrition in toddlers can be done through the diversification and development of supplementary food formulas, considering aspects of nutrition, health benefits, acceptability, shelf-life, and the advantages of local food resources. Meeting the nutritional needs of toddlers can be achieved by increasing the intake of nutritious foods, such as catfish and spinach (Untari *et al*., 2022).

Catfish contains 17% protein, 6.6% fat, 0.23 – 1.3% carbohydrates, 0.9% ash, and 74.4% water. Compared to the fat content of other freshwater fish such as snakehead fish (4.0%) and carp (2.9%), catfish has a higher fat content (Nurfajrina & Hastuti, 2021). Spinach is rich in calcium, iron, vitamin A, B, E, and C, fiber, and beta-carotene. It also has high mineral content, especially iron, which can be utilized to combat stunting (Suciati *et al*., 2020).

Based on the background, the researchers conducted a study aimed at creating a functional food formula using catfish and spinach flour, also analyzing the macronutrient and iron content of the biscuits. With the development of this biscuit formula, we hope to create a functional food product that can contribute to stunting prevention efforts. Fortified biscuits made with catfish and spinach flour are expected to support government initiatives aimed at preventing stunting.

2. Methods

2.1. Sample preparation

The research was conducted at the Food Technology Laboratory and the Chemistry Laboratory of the Nutrition Departement, Poltekkes Kemenkes Palangka Raya. The research was conducted from January to March 2024. The sample preparation stage consists of making catfish flour, spinach flour, and the formulation of the biscuit.

2.1.1. Preparation of catfish flour

The procedure for processing catfish flour was based on the method used by Suciati *et al*. (2020), with slight modifications. One kilogram of cleaned catfish was weighed. The weighed catfish was seasoned with lime juice and salt and steamed for approximately 10 minutes. After that, to remove excess fat, the catfish was crushed and squeezed using cloth. The crushed catfish was dried in a dehydrator at 40°C for 10 hours. The dry catfish was ground using a blender, mixed with wheat flour until evenly mixed, and sifted with an 80 mesh sieves.

2.1.2. Preparation of spinach flour

The procedure for processing spinach flour followed the method used by Machfudloh *et al*. (2019) with modification. The spinach leaves were cleaned and separated from the stems. 300 grams of spinach leaves were dried using dehydrator at 60°C for 19 hours. The dried spinach leaves were ground using a blender for 5 minutes a sifted with an 80-mesh sieve.

2.1.3. Formulation of catfish and spinach biscuits

In this study, biscuits were made with three formulas and using 10 kinds of ingredients as shown in **Table 1** (Mohamad *et al*., 2022).

Table 1. Biscuit formulations

Ingredients	F1	F2	F3
Wheat flour	240 g	240 g	240 g
Catfish flour	15 g	30 g	45 g
Spinach flour	45 g	30 g	15 g
Milk powder	10 g	10 g	10 g
Margarine	50 g	50 g	50 g
Baking powder	3 g	3 g	3 g
Vanilla	3 g	3 g	3 g
Salt	3 g	3 g	3 g
Sugar	65 g	65 g	65 g
Egg	1	1	1

The procedure for making biscuits followed the method used by Suciati *et al*. (2020). All ingredients were weighed according to the formula of each biscuit and mixed thoroughly until well combined. The dough was then poured into a mold then baked in an oven at 100°C for 35 minutes.

2.2. Method and result analysis

The biscuits were analyzed for protein, carbohydrate, water, and ash content. These tests followed the method that used by Annisa & Suryaalamsah (2023), Bolang *et al*. (2022), Pebrina *et al*. (2021), Yudhistira *et al*. (2019) with minor modifications. Protein content was analyzed using Kjeldahl method, carbohydrates were measured using Titrimetric method, and fat content was

measured using Soxhlet extraction method. Water and ash content were analyzed using Gravimetric method. Iron content was assessed using Atomic Absorption Spectroscopy (AAS).

2.2.1. Protein analysis

As many as 2 grams of biscuit samples were placed in a 100 mL Kjeldahl flask, along with 2 catalyst tablets and some boiling stones. Then, 15 mL of H_2SO_4 (95%-97%), and 3 mL of H_2O_2 were slowly added, and the solution was allowed to stand for 10 minutes in an acid room. Destruction was performed at 410°C for about 2 hours until the solution became clear. Cooled the samples to room temperature and diluted with 50-75 mL of distilled water.

Afterward, 25 mL of 4% boric acid solution was added into an Erlenmeyer flask. The flask containing the destruction result was placed in a stem distillation apparatus, and 50-75 mL of sodium hydroxide thiosulfate solution was added. The distillate was collected in the Erlenmeyer flask containing the 4% boric acid solution until it reached a volume of at least 150 mL. The distillate was then titrated with standardized 0.2 N HCl until the color changed from green to neutral grey.

2.2.2. Fat analysis

An empty round-bottom flask was weighed for fat analysis. Two grams of biscuit sample were placed in a Soxhlet extractor and destructed at 60°C for 8 hours. The fat and chloroform mixture in the flask were evaporated until dry. The round-bottom flask containing the fat was placed in an oven at 105°C for 2 hours. The flask and fat were cooled in a desiccator for 30 minutes.

2.2.3. Carbohydrate analysis

Ten milliliters of biscuit sample solution was pipetted into an Erlenmeyer flask. Then, 10 mL of Fehling's A and B solution and 2-4 drops of 0.2% methylene blue were added. The mixture was heated on a hot plate magnetic stirrer. Once boiling, it was titrated with a standard sugar solution until the blue color disappeared. The titration was performed quickly.

2.2.4. Moisture content analysis

Two grams of finely ground biscuit sample were placed in a pre-weighed porcelain crucible. The sample was dried in an oven at 100-150°C (3-5 hours). After that, the sample was cooled in a desiccator and weighed. The sample was redried in the oven for 30 minutes, cooled, and weighed again. This process was repeated until a constant weight achieved.

2.2.5. Ash content analysis

An empty porcelain crucible was burned in a furnace at 550°C for 1 hour. The crucible was dried in an oven at 100-105°C for an hour, cooled in a desiccator for 15 minutes, and weighed until a constant weight was achieved. Next, 5 grams of biscuit were placed in the crucible and ash in the furnace at 550°C for 4 hours. The ash sample was dried in an oven at 100-105°C for an hour, cooled

in a desiccator for 15 minutes and weighed. The process was repeated until a constant weight was achieved.

2.2.6. Iron (Fe) analysis

The iron content of the biscuit samples was analyzed using Atomic Absorption Spectroscopy (AAS) with 510 nanometers wavelength.

3. Result and discussion

The nutritional content of the biscuits was tested using proximate analysis. The results of the analysis of the biscuits with catfish flour and spinach flour substitution are presented in **Table 2**.

Table 2. Results of moisture, ash, carbohydrate, protein, and fat content testing of formula F1, F2, and F3

No.	Chemical analysis	Results		
		F1 (mean ± SD)	F2 (mean ± SD)	F3 (mean ± SD)
1.	Protein (%)	9.74 ± 0.366	10.44 ± 0.087	11.03 ± 0.083
2.	Fat (%)	31.217 ± 0.253	30.939 ± 0.779	31.073 ± 0.512
3.	Carbohydrate (%)	43.08 ± 0.250	43.37 ± 0,193	43.13 ± 0,184
4.	Moisture content (%)	3.75 ± 0.013	2.08 ± 0.022	2.26 ± 0.017
5.	Ash content (%)	3.34 ± 0.029	2.93 ± 0.072	2.98 ± 0.057
6.	Iron (%)	7.157 ± 0.11	6.530 ± 0.08	5.025 ± 0.09

The protein content analysis for the three biscuit formulas, as presented in **Table 2**, shows that all biscuits have a protein content above 5%. According to SNI 2973-2011, the minimum protein requirement for biscuits is 5%. Protein is essential for the body to maintain normal metabolic processes. Both animal and plant proteins contain amino acids that help build and repair body tissues, form hormones and enzymes, and serve as an energy source (Sidoretno *et al*., 2022).

Insufficient consumption of protein-rich foods can lead to a protein energy deficiency in children (Untari *et al*., 2022). A fast-acting approach to addressing stunting is encouraging the continuous consumption of animal protein sources, which are high in protein and essential amino acids (Eliana *et al*., 2022). Fish, including catfish, is a rich source of amino acids (Handarini & Madyowati, 2021).

According to SNI 01-2973-1992, the minimum fat content required in biscuits is 9.5 g/ 100 g (Loppies *et al*., 2021). As shown in **Table 2**, all the biscuit formulas meet this standard, with fat content percentages exceeding 30%. Fat is necessary for the body to function properly and aids in the absorption of vitamins A, D, E, and K. A lack of fat can lead to vitamin deficiencies (Sidoretno *et al*., 2022). Research conducted by Pandiangan (2021) indicates that catfish contains omega-3 and omega-6 fatty acids, which are beneficial for human health, including children's health (Pandiangan, 2021). Fish contains omega-3 fatty acids, which are polyunsaturated essential fatty acids crucial for brain development in children (Handarini & Madyowati, 2021).

The carbohydrate content of biscuits with formulas 1, 2, and 3 are 43.08, 43.37, and 43.13%, respectively. According to SNI (1992), the minimum carbohydrate content required for biscuits is 70%, meaning that these biscuits do not meet the carbohydrate quality standards set by SNI. Carbohydrates serve as the body's primary energy source (Sidoretno *et al*., 2022). Ingredients in biscuits that contain carbohydrates include wheat flour, milk, and sugar (Gita & Danuji, 2018). To increase the carbohydrate content of biscuits, it is suggested to increase the proportion of wheat flour and powdered sugar in the biscuit dough. Wheat flour consists of 67-70% carbohydrates (Annisa & Suryaalamsah, 2023).

SNI 01-2973-2011 states that the maximum moisture content in biscuits should be 5%. The water content analysis for three biscuit formulas shows that all biscuits (F1, F2, and F3) have moisture levels below 5%. Moisture content is crucial for determining the freshness and shelf-life of food products. High moisture content promotes the growth of microorganisms, leading to faster spoilage (Sidoretno *et al*., 2022).

The ash content of the biscuit formulas, as presented in **Table 2**, exceeds the maximum allowed by SNI, which is 2%. Ash content measures the mineral content in food (Sidoretno *et al.*, 2022). The high ash content in these biscuits is influenced by the mineral-rich ingredients used, particularly spinach. Spinach contains high levels of minerals, such as calcium, iron, and vitamins A, B, E, and C (Suciati *et al*., 2020).

The average iron content found in the biscuits was as follows: formula F1 = 7.157 mg/ 100 g, formula F2 = 6.530 mg/ 100 g, and formula F3 = 5.025 mg/ 100 g. The biscuit formula with the highest iron content was F1. Based on The Nutritional Adequacy Rate for Indonesian People, the iron intake requirement is 7 – 8 mg per day (Kemenkes RI, 2019). Consuming 100 g of formula F1 biscuit can meet the iron requirement per day.

Green spinach is rich in iron, which is essential for red blood cell production. Adequate red blood cell production helps prevent anemia. Iron-deficiency anemia increases the risk of low birth weight (LBW), reduced iron stores in infants, and can disrupt brain growth and development (Sulistyaningrum & Fitriyanti, 2022). Fresh spinach contains 2.71 mg of iron per 100 grams (Falah AS *et al*., 2023). The higher iron content in the biscuits compared to fresh spinach may be due to the additional iron present in catfish. Catfish contains approximately 1.6 mg of iron per 100 grams (Sulistyaningrum *et al.*, 2021). Fish consumption can also help improve iron absorption (Handarini & Madyowati, 2021).

4. Conclusion

Based on nutritional calculations, it was found that biscuit with formula F3 met the minimum requirements of SNI regarding moisture, protein, and fat content. However, it did not meet the minimum standards of carbohydrate, ash, and iron content (except formula F1). Further research should modify the biscuit formula by adding the concentration of spinach flour in the formula and combining the formula with wheat flour, so that the biscuit can meet the minimum standards of carbohydrate, ash, and iron content.

Acknowledgement

We would like to express our gratitude to Poltekkes Kemenkes Palangka Raya for facilitating this research, providing financial support, and offering infrastructure and resources.

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