

Development of Omega-3 and Antioxidant-Rich Bread Spread from Smoked Mackerel and Bambara Groundnut for Cardiovascular Health

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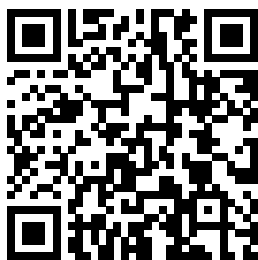
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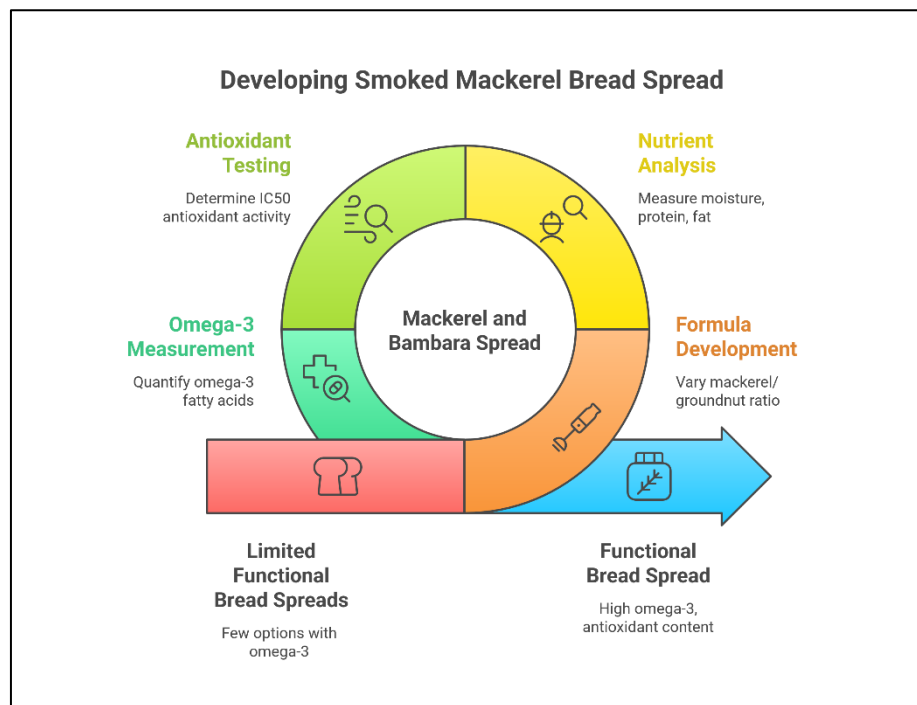
ABSTRACT

Bread spreads can be defined as products that can be spread on bread or as a filling, usually used to add flavor or texture to bread. The taste of bread spread is not only sweet but can be spicy and savory. The purpose of this research was to develop a bread spread made from smoked mackerel with the addition of bambara groundnut and analyze the effect of different formulas on nutrient content, antioxidant activity, and omega-3. This study used a completely randomized design. The analysis test used was One-Way ANOVA with Duncan multiple range test further test with SPSS version 26 and Microsoft Excel 2016 software. The results of this study showed that the highest content of moisture, protein, fat in F1, ash content in F2, while carbohydrates, energy, IC50, and omega-3 in F3. The results of the analysis on bread-spread products contained moisture 31.57-41.06%, ash 4.56-5.00%, protein 15.84-26.82%, fat 28.30-36.24%, carbohydrate 32.14-51.31%, IC50 is 2.66-4.32-ppm, and omega-3 of 1.68-2.6 g/100g. The conclusion of the research showed that the treatment of the ratio of mackerel and bambara groundnut affected on the moisture, protein, and carbohydrate ($p < 0.05$), but did not affect on the ash, fat, omega-3, and antioxidant activity ($p > 0.05$).

Key Messages:

- The product of bread spread can be used as a functional food containing antioxidants and omega-3 fatty acids as anti-inflammatories.
- Researchers have yet to develop a bread spread product made from smoked mackerel with the addition of Bambara groundnut as a functional food.

GRAPHICAL ABSTRACT



INTRODUCTION

Coronary heart disease (CHD) is one of the most common types of cardiovascular disease. Cardiovascular disease refers to disorders of the heart and blood vessels, including stroke, rheumatic heart disease, and other conditions (1). Global statistical data show that cardiovascular diseases caused 17.9 million deaths annually in 2019, accounting for 32% of all global deaths (2). It is estimated to increase to 23.3 million by 2030 (3). According to the 2018 Riskesdas data, the prevalence of heart disease in Indonesia based on doctor diagnosis is 1.5%. In comparison, the prevalence of heart disease in West Java province is higher, reaching 1.62% (4). Nutrient intake is the most important factor in preventing premature death from cardiovascular disease, even more so than not smoking and physical activity (5).

Polyunsaturated fatty acids (PUFAs) are essential fatty acids for humans. There are two main PUFAs obtained from food: omega-3 fatty acids and omega-6 fatty acids. The primary omega-3 fatty acids include DHA, EPA, and ALA (6). Omega-3 fatty acids reduce mortality from cardiovascular disease (7). Omega-3 plays a role in preventing cardio-metabolic diseases, so it is recommended to increase its intake in the diet (8). Consumption of fish or foods rich in omega-3 fatty acids is an important component of a heart-healthy diet, and certain individuals may benefit from omega-3 fatty acids for cardiovascular health (9).

Mackerel is one of the small pelagic fish species with significant economic value and is a primary catch. In addition to its economic value, mackerel is a highly sought-after natural commodity and a potential natural resource for exploitation (10). Mackerel has a high nutritional content, with protein levels ranging from 17-23% (11). Mackerel contains 70% omega-3 fatty acid of the total fat in mackerel (Effendie 2002; Suroso et al. 2018) and omega-6 fatty acids (12), which are beneficial for immune system function, memory, vision, and mental health (13). Mackerel is one of the food sources with good nutritional content to meet a wide range of health needs. According to data from the Ministry of Health in the Indonesian Food Consumption Table, 100 g of mackerel or Banjar fish contains protein, fat, carbohydrate, and various minerals. Meanwhile, Bambara groundnut also contain omega-3 and antioxidant compounds, which, when combined with mackerel, can provide synergistic effects on heart health.

Bambara groundnut (BGN) are one of the legume plants that can grow in Indonesia (14). One of the minor legumes that is less noticed in Indonesia is the BGN. The botanical name of the BGN plant is *Vigna subterranea* (L) Verdc. BGN are commonly found around Bogor and other areas in West Java. The BGN are

traditionally cultivated by farmers, with various varieties originating from seeds produced by their own plants (15). BGN contain polyphenolic compounds, polyunsaturated fatty acids, several minerals (calcium, potassium, and iron), and phytochemicals. Despite containing numerous phytochemicals with high biological value, the components of BGN have not yet been utilized as nutraceutical and functional food development ingredients (6).

Bambara groundnut are highly nutrient-dense and are referred to as a “complete food” due to their balanced macronutrient content, making them a suitable source of protein. Bambara groundnut contain both nutrients and antinutrients. By using standard food processing methods, the antinutrients in BGN and other leguminous crops can be successfully reduced or removed (6). Soaking aims to reduce anti-nutritional factors (oxalate and phytic acid) and can improve the quality of nutrients and minerals (calcium, potassium, and iron) in the seeds (16). Bambara groundnut have been shown to contain omega-3 and omega-6 fatty acids (6).

Additionally, not only is mackerel high in protein, but it also contains omega-3 fatty acid, which have been shown to lower the risk of cardiovascular disease. BGN are also a good source of antioxidants. Combining the two into a bread spread makes this product innovative, highly nutritious, and simple to include in regular diet. Mackerel and BGN are made into bread spreads for practical, easy-to-consume and popular among people of all age groups, especially adults who needs a balanced diet but have busy lifestyles. According to Wicaksono et al. (2025), it is explained that unhealthy lifestyle habits among the general population, including those within academic communities, can raise the risk of CHD and negatively impact quality of life (17).

Based on the above data, there is a need for a method to utilize local foods, particularly Bambara groundnut and smoked mackerel, into practical processed products that have the potential to improve cardiovascular health. Some bread spreads are protein-based, such as those made from tuna and chicken; however, researchers have not conducted studies on the development of bread spreads made from mackerel with the addition of Bambara groundnut. Therefore, research is needed on the development of mackerel bread spreads with the addition of Bambara groundnut as an alternative functional local food.

METHODS

Design, Place, and Time of Research

This research is an experimental laboratory with a Complete Randomized Design (CRD) with a single factor, namely the composition of mackerel and bambara groundnut in three different treatment levels with two replication levels. Mathematical model and design this research is as follows:

$$Y_{ij} = \mu + A_i + \epsilon_{ij}$$

Y_{ij} : experimental response due to the effect of the addition of bambara groundnut at level 1, replication j

μ : mean of value

A_i : effect of the combination of mackerel and bambara groundnut

ϵ_{ij} : research error due to the effect of the i level of bambara groundnut addition in the j replication

: number of treatments combining mackerel and bambara groundnut (i = 1, 2, 3)

: number of replication (j = 1, 2)

The bread spread formula was determined through several trials, resulting in a comparison of the ratio of mackerel and Bambara groundnut, comprising three formulas: F1 (75:25), F2 (50:50), and F3 (25:75). The determination of these formulas was based on the research by Karnjanapratum et al. (2022) (18).

This research was conducted from July to November 2024 with two stages. The preliminary stage involved developing the bread spread formulations at the Food Experiment Laboratory, Departemen Gizi Masyarakat FEMA-IPB. Before making the product, the mackerel was cold-smoked at the Ilmu Teknik Kelautan FPIK-IPB, and the bambara groundnut was dried at the Seafast Center IPB. The advanced stage was carried out by conducting proximate analysis and antioxidant activity tests at the Chemistry Laboratory of the Departemen Gizi Masyarakat FEMA-IPB. Proximate analysis consisted of analysis of moisture, ash, fat, and protein content. Omega-3 analysis of the product at Terpadu Laboratory IPB.

Materials

The tools used in the research consist for making bread spreads, preference tests, and chemical analysis. The tools used for proximate testing include an analytical balance, porcelain dishes, aluminum cups, electric hot plates, stoves, ovens, desiccators (Daihan, WBA-220, Korea Selatan), digestors (FOSS, TM 2508, Denmark), distillers, pH meters (Ohaus, Starter-3100, Amerika Serikat), burettes (Pyrex, Prancis), stands, beakers, Erlenmeyer flasks, Soxhtec (FOSS ST-243, Denmark), soxtec *control unit* (CU-2046, Denmark), Kjeltex (FOSS, KT-200, Denmark), pipettes, filter paper (Hawach, BIO-41-11, Cina), Buchner funnels, measuring cups, and graduated cylinders, a set of gas chromatography (Shimadzu, GC-2010 Plus, Jepang), and a set of spectrophotometer UV-Vis (Shimadzu, UV-1800, Jepang). The ingredients used in making fish bread spread are mackerel, bambara groundnut, salt, seasoning, lime, mayonnaise, pepper, and oil. Materials used in the proximate test include bread spread samples.

Analysis Procedures

The mackerel used in this study were smoked mackerel that had been filleted and separated from the bones, purchased from a supermarket. The specifications of the mackerel were determined by the researcher based on the standards for fresh fish according to SNI 2729:2013 (SNI 2013) with the following details: whole, bright eyes, fresh smell, firm texture, elastic, and compact. Lime juice can neutralize the fishy odor in fish and shellfish within 30–60 minutes. The smoking process for fish has been proven to reduce lipid oxidation levels and significantly inhibit autoxidation during storage. Smoked fish significantly reduces the loss of EPA and DHA content (19). The temperature used in cold smoking refers to SNI 9296:2024 on Cold-Smoked Fish, which specifies 50 °C for 8 hours to reduce moisture content and impart a smoky aroma to the fish meat.

The Bambara groundnut used in the study were Bambara groundnut that had been separated from their shells. The specifications of the Bambara groundnut were determined by the researchers, namely raw Bambara groundnut with the following details: red-purple-black seeds, undamaged, free of worms, free of holes, and intact. The process of making Bambara groundnut involves separating them from their outer shell, washing them with running water, and then soaking them in a solution of 1% NaHCO₃ and 1% NaCl for 24 hours to reduce antinutritional factors (16). After that, the beans are washed, drained, and boiled for 15 minutes (20) at a temperature of 90–100 °C or gently simmered, filtered, drained, and placed in a grinder to break the Bambara groundnut, which are then placed in a drum dryer at a temperature of 110 °C at a speed of 1.15 rpm (21). After the Bambara groundnuts are ground into flour, they are sieved using a 50-mesh sieve.

The research data were processed using Microsoft Excel 2016 and IBM SPSS version 26 also analyzed using One-Way ANOVA with Duncan's Multiple Range Test at a 5% level. Proximate analysis includes moisture content analysis (Gravimetric method, AOAC 2006) (22), ash content analysis (Gravimetric method, AOAC 2006), protein content (Kjeldahl method, AOAC 2006) (22), fat content (Soxhlet method, AOAC 2006) (22), and carbohydrate content (by difference), antioxidant analysis (DPPH) (23,24), and omega-3 fatty acids were performed using gas chromatography (GC-FID type 2010 method, BSN 1992 & AOAC 2012). The following is a flowchart of the bread spread making process in Figure 1.

Based on the flowchart in Figure 1, smoked mackerel and processed Bambara groundnut are mixed and minced together with other ingredients. The mixture is pasteurized by controlling the temperature at 73±2 °C and holding it for 15 minutes. The mixture is then packaged in sterile glass bottles. The sample is stored at room temperature (between 20–25 °C) until the core temperature reaches 23±2 °C, and then proximate analysis is performed (18).

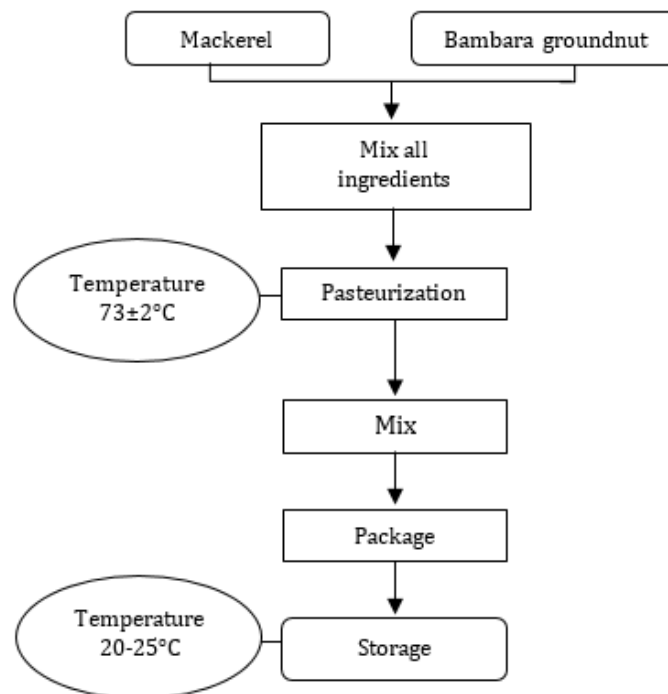


Figure 1. Flowchart of the bread spread-making process

RESULTS

The bread spreads formulated from smoked mackerel and bambara groundnut contain moisture, ash, fat, protein, and carbohydrates. The nutritional content of bread spreads can be seen in Table 1.

Table 1. Nutrient content of bread spread made from mackerel and bambara groundnut (%)

Parameter	Formula			p-value
	F1(75:25)	F2(50:50)	F3(25:75)	
Moisture content	41.06±0.48 ^c	36.48±0.13 ^b	31.57±0.14 ^a	0.000*
Ash content	4.80±0.12	5.00±0.28	4.56±0.19	0.249
Fat content	36.24±3.03	30.44±.50	28.30±1.85	0.188
Protein content	26.82±0.39 ^c	19.20±1.05 ^b	15.84±0.20 ^a	0.001*
Carbohydrate Total	32.14±2.52 ^a	45.34±3.19 ^b	51.31±2.23 ^b	0.012*

Note: The formula is the ratio of mackerel to bambara groundnut (%). All nutrient content is dry weight, except moisture content. Statistical analysis was performed using ANOVA followed by Duncan's test. Values with different letters indicate significant differences ($p < 0.05$).

The measurements in Table 1 above are based on dry weight. The moisture content of bread spreads made from mackerel and Bambara groundnut ranges from 31.57–41.06%. The ANOVA analysis's findings show that the ratio of mackerel and Bambara groundnut significantly affects the moisture content of the bread spread product. The Duncan post-hoc test results show there are highly significant differences between the formulas. Bread spreads have an ash content of 4.56 to 5.00%. The ash content of bread spread product is not significantly impacted by the treatment ratio of mackerel and Bambara groundnut, according to the ANOVA analysis. Bread spread has a total fat content of 28.30–36.32%. According to the analysis findings, the total fat content of bread spread products is not substantially impacted by the treatment ratio of mackerel to Bambara groundnut.

The bread spread's protein level varied between 15.84 and 26.82%. the protein content of the bread spread product was considerably impacted by the proportion of mackerel and Bambara groundnut, according to result of the ANOVA analysis. A highly significant difference in the formulation was revealed by the Duncan post-hoc test. The range of the bread spread items total carbohydrate content was 32.14–

51.31%. Based to the ANOVA results, the bread spread product's carbohydrate content is significantly influenced by the proportion of mackerel to Bambara groundnut. Formula differences are substantial, according to the Duncan post-hoc test.

In addition to performing proximate analysis to determine the nutritional composition of the product, this research also involved antioxidant activity analysis expressed in IC_{50} values to evaluate the potential of bioactive compounds in neutralizing free radicals. The results of the analysis in Figure 2 show the antioxidant capacity of extracts from mackerel bread spread and Bambara groundnut.

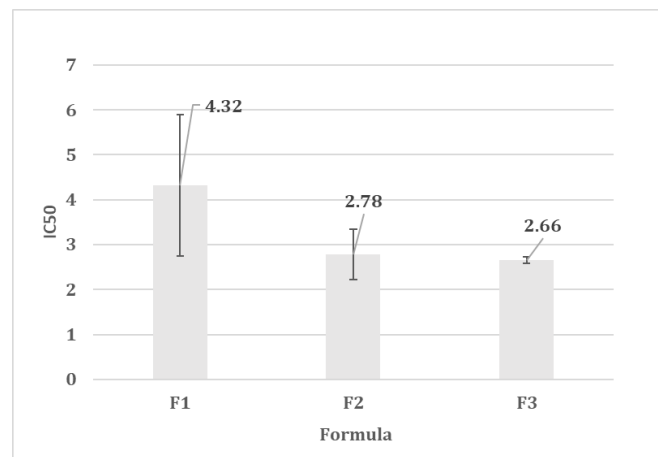


Figure 2. The effect of the ratio of mackerel and Bambara groundnut on the IC_{50} of bread spreads

The ANOVA results in Figure 2 show that the treatment of mackerel and Bambara groundnut ratios had no significant effect on the antioxidant activity of the bread spread products. Although there was no significant effect, the analysis results showed that all formulas had IC_{50} values <50 ppm, which means that the antioxidant activity in the bread spread products was very strong according to Molyneux et al. (2004). Antioxidant activity is classified as “very strong” if the IC_{50} value is less than 50 ppm, “strong” if the IC_{50} value is 50–100 ppm, “moderate” if the IC_{50} value is 101–150 ppm, and “weak” if the IC_{50} value is 150–200 ppm. Antioxidants are compounds with molecular structures that can freely donate electrons to free radical molecules, thereby interrupting and inhibiting the oxidative reactions of free radicals.

The research also analyzed the omega-3 fatty acid content, including ALA, EPA, and DHA, to determine the product's functionality in supporting health, particularly in the prevention of degenerative diseases such as coronary heart disease. The results of the omega-3 content analysis in bread spreads are presented in Table 2.

Table 2. The effect of mackerel and Bambara groundnut ratio on omega-3 fatty acid content in bread spreads (g/100 g)

Parameter	Formula			p-value
	F1(75:25)	F2(50:50)	F3(25:75)	
Omega-3	1.68±0.66	2.11±0.16	2.26±0.14	0.433
Alpha-linolenic acid (ALA)	0.06±0.00	0.06±0.01	0.07±0.02	0.269
Eicosapentanoic acid (EPA)	1.51±0.97	1.40±0.25	0.85±0.56	0.561
Docosahexaenoic acid (DHA)	0.55±0.43	0.50±0.99	0.31±0.06	0.662

Note: The formula is the ratio of mackerel to bambara groundnut (%). Statistical analysis was performed using ANOVA. Values with different letters indicate significant differences ($p < 0.05$).

Based on the results in Table 2, the omega-3 fatty acid ranged from 1.68 to 2.26 g, ALA ranged from 0.06 to 0.07 g, EPA ranged from 0.85 to 1.51 g, and DHA ranged from 0.31 to 0.55 g. The ANOVA results indicate that the treatment ratio of mackerel and Bambara groundnut does not significantly affect the omega-3, ALA, EPA, and DHA content of the bread spread product. The highest EPA and DHA content is found in formula F1, which contains a high proportion of mackerel. The high of EPA and DHA content is thought to be caused by the processing of mackerel, namely by cold smoking.

DISCUSSION

Data from the Ministry of Health in the Indonesian Food Consumption Table shows that 100 g of mackerel or Banjar fish contains 111 kcal of energy, 19.5 g of protein, 0.9 g of fat, 4.8 g of carbohydrates, 68 g of ash, 68 mg of calcium, 278 mg of phosphorus, 7.3 mg of iron, and 339 mg of potassium. Meanwhile, 100 g of dried Bambara groundnut contains 370 kcal of energy, 16 g of protein, 6 g of fat, 65 g of carbohydrates, 85 mg of calcium, 264 mg of phosphorus, 4.2 mg of iron, and 868.8 mg of potassium.

According to Table 1, formula F3 has a lower moisture content compared to the other formulas, likely due to a higher proportion of Bambara groundnut flour. The moisture content of this bread spread is lower than the other bread spreads such as catla fish bread spread (25) and chicken bread spread (26). Bambara groundnut flour has a lower moisture content than smoked mackerel. The results of Jayanti et al. (2024) showed that the average moisture content of smoked fish using the cold smoking method was 25.53–32.45% (27). Bread spreads made from catla fish with mangrove flower buds have a moisture content of 72.50% (28). The moisture content of bread spread products made from chicken with silkworm pupae is 58.07–64.81% (18). Moisture content plays a role in influencing product quality and shelf life. Moisture content is also a factor in determining product texture quality.

Ash content analysis is used to detect the mineral content in food ingredients by burning the organic components of the food. High ash content affects the mineral content in food ingredients. The ash content (Table 1) in the bread spread varied between 4.56% and 5.00%. All formulations did not differ significantly according to statistical analysis, suggesting that changes in product composition had no impact on ash concentration of the bread spread. The smoking process contributes to changes in the mineral content of fish, including an increase in potassium and a decrease in copper levels in mackerel (29). Although not significantly different, this product has a higher ash content compared to chicken bread spreads with ranges from 1.51 to 2.18% (18).

Formula F1 on Table 1 had a higher protein content compared to the other formulas; it is 26.82%, likely due to its higher mackerel content. Mackerel has a relatively high nutritional content, with a protein content of 17–23% (11). Formula F1 has a significant difference from formulas F2 and F3. Formula F3 has a higher carbohydrate content compared to the other formulas, likely due to its higher composition of Bambara groundnut flour. Bambara groundnut flour has a higher carbohydrate content than smoked mackerel. Bambara groundnut has a high nutritional value in carbohydrate content ranging from 55–71% (30) and in Bambara groundnut flour, it is 61.17% (31). This smoked mackerel bread spread with Bambara groundnut has a much higher carbohydrate content than chicken bread spread with silk worm cocoons, which ranges from 5.2–11.14% according to Karnjanapratum et al. (2022) (18).

Antioxidant levels determine a food's nutritional and functional value (32). Based on Figure 2, although not significantly different, the analysis results show that all formulas have an IC_{50} value <50 ppm, which means they are very strong. It can be concluded that formula F3 has greater activity or the capacity to inhibit free radical because its IC_{50} value is smaller than the others, at 2.66 ppm. This is confirmed by Maphosa and Jideani (2016), that antioxidants compounds have the ability to react with free radicals, forming stable or non-reactive radicals, thereby reducing inflammation in the human body (30). A diet high in antioxidants can reduce oxidative stress and inflammation, as well as reduce the risk of cardiovascular disease (33). A diet rich in antioxidants has the potential to improve cardiovascular health and prevent cardiovascular disease (34).

Based on the results of proximate analysis and other nutritional content, the formula with the best tendency was determined to be F1. This smoked fish and bambara groundnut spread product contains 331 kcal of energy, 26.82 grams of protein, 36.24 grams of total fat, 32.14 grams of carbohydrates, 1680 mg of omega-3, 60 mg of ALA, 1510 mg of EPA, and 550 mg of DHA per 100 grams. This research has limitations in terms of its effectiveness in preventing coronary heart disease. Although it was formulated for the prevention of CHD in adults, clinical trials have not yet been conducted to prove its direct benefits. This study is likely still at the laboratory scale, so it has not yet considered challenges in mass production, such as cost efficiency and product durability. Potential bias may arise in sensory tests because panelists' assessments are influenced by individual preferences, prior experiences, and conditions at the time of testing.

CONCLUSION

Bread spread products have contents of moisture, ash, total fat, protein, and carbohydrate that are various depending on the ratio of formulation. The results of the study indicate that the highest levels of moisture, protein, fat, EPA and DHA are found in formula F1, the highest ash content in formula F2, while carbohydrates, antioxidants, omega-3 and ALA are highest in formula F3. The analysis results indicate that the comparison treatment of smoked mackerel and Bambara groundnut significantly affects moisture content, protein, and carbohydrate levels ($p < 0.05$), but does not significantly affect ash content, fat content, antioxidant activity, and omega-3 levels (including ALA, EPA, and DHA) ($p > 0.05$).

In further research, it is necessary to test the total number of microorganisms that may contaminate the product to ensure better quality, as well as the acceptance of the bread spread product by the target market. Additionally, shelf life testing is required to determine the product's shelf life to ensure it can be consumed in good condition. An analysis of commercial potential also needs to be conducted to evaluate the opportunity for this product to be marketed more widely, however the product needs to be reformulated to improve texture, spread-ability and appearance.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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