



Evaluation of macro nutrient, fatty acid and microbiology aspect of mackerel tuna crackers

Evaluasi gizi makro, asam lemak dan aspek mikrobiologi kracker ikan tongkol

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Abstract

Stunting is a health issue related to chronic malnutrition over 1000 days of life. Fulfilling the nutritional needs of pregnant women through fish consumption has been recommended in several studies. In Indonesia, mackerel tuna is a popular fish type at affordable prices. We developed mackerel tuna crackers as a nutritional product innovation for pregnant women made from mackerel tuna meat, eggs, and various spices. This product was developed as a food supplement for pregnant women. This study aimed to evaluate the macronutrients, fatty acids, and microbiological aspects of the product. Laboratory analyses were performed at the Integrated Laboratory of the IPB University. The results showed that the protein content of the product was highest in formula 1 (39,71%), meeting 54% of the protein requirement of pregnant women according to the IOM 2005 recommendation and 44,12% protein requirement of pregnant women according to AKG 2019. The total omega-3 content was highest in formula 2 (1,18%), and the total omega-6 content was highest in formula 3 (10,78%). The results of the microbiological test showed that all product samples were safe according to SNI 7988:2009. Further studies on the efficacy of mackerel tuna crackers as a nutritional intervention in pregnant women are urgently required.

Keywords: Fatty acid, food supplementation, mackerel tuna crackers, pregnant women

Abstrak

Stunting merupakan salah satu masalah kesehatan yang berkaitan dengan kekurangan gizi kronis pada 1000 hari kehidupan. Memenuhi kebutuhan gizi ibu hamil dengan mengonsumsi ikan telah direkomendasikan oleh beberapa penelitian. Di Indonesia, ikan tongkol merupakan salah satu jenis ikan yang digemari dan harganya relatif terjangkau. Kami telah mengembangkan kraker ikan tongkol sebagai inovasi produk untuk dikonsumsi ibu hamil yang terbuat dari daging ikan tongkol, telur, dan berbagai bumbu. Produk ini dikembangkan untuk menjadi makanan tambahan bagi ibu hamil. Tujuan dari penelitian ini adalah untuk mengevaluasi kandungan zat gizi makro, asam lemak, dan aspek mikrobiologi produk. Analisis laboratorium dilakukan di laboratorium terpadu, IPB University. Hasil penelitian menunjukkan bahwa kandungan protein produk tertinggi terdapat pada formula 1 (39,71%), angka ini dapat memenuhi 54% kebutuhan protein ibu hamil menurut rekomendasi IOM 2005 dan dapat memenuhi 44,12% kebutuhan protein ibu hamil menurut AKG 2019. Total kandungan omega-3 tertinggi terdapat pada formula 2 (1,18%) dan total kandungan omega-6 tertinggi terdapat pada formula 3 (10,78%). Hasil uji mikrobiologi menunjukkan bahwa semua sampel produk aman berdasarkan standar SNI 7988:2009. Diperlukan penelitian lebih lanjut terkait efikasi konsumsi kraker ikan tongkol terhadap status gizi ibu hamil.

Kata Kunci: Asam lemak, ibu hamil, kraker ikan tongkol, suplementasi makanan

Introduction

Approximately 150 million children below five-year old around age world have experienced stunting. This condition of malnutrition stems from systemic food failure, characterized by low affordability and access to nutritious food (WHO and Bank 2023). Indonesia is among the countries that are facing this stunting issue. Although the number of stunting cases in Indonesia has decreased to 21% by 2022, the prevalence of stunting in several provinces and municipalities remains significantly high. Malnutrition during pregnancy is positively correlated with children's anthropometric status (Haque et al. 2022).

Pregnancy is a critical phase that significantly affects the nutritional status of new-borns. The role of the mother during this period is crucial in preventing stunting (Saleh et al., 2021). Maternal nutritional intake during pregnancy significantly affects the incidence of stunting in children (Fitriani et al. 2020). Nutritional intervention in mothers during pregnancy can improve fetal, neonatal, and infant outcomes (Gonz et al. 2024). During pregnancy, protein requirements increase by approximately 1 g/day in the first trimester, 8 g/day in the second trimester, and 26 g/day in the third trimester, which must be met to support fetal and placental growth and development (Marangoni et al. 2016).

Chronic maternal energy deficiency (CED) is a major nutritional concern during pregnancy. Based on data from the 2023 Indonesian Health Survey (IHS), the prevalence of pregnant women at risk of CED in Indonesia was 16,9%, a 10,6% increase from the 2018 RISKESDAS (Badan Penelitian dan Pengembangan Kesehatan (Badan Litbangkes) 2018; Kementerian Kesehatan RI 2023). The prevalence was high and far exceeded the WHO Health Organization tolerance standard of 5%. Inadequate nutritional intake is the main cause of CED. Only 32,1% of pregnant women with CED received supplementary feeding. This high rate of CED contrasts with Indonesia's abundance of natural resources, which can be harnessed to provide nutrient-rich food to meet family nutritional needs, particularly for pregnant women. Various local food sources, especially those rich in protein, have the potential to enhance the nutritional supply of pregnant women with CED.

Mackerel tuna (*Euthynnus Affinis*) is an excellent protein source that is comparable to other commercially significant tuna species, such as skipjack, yellowfin, and bigeye tuna (Kannaiyan et al., 2019). Mackerel tuna is affordable and nutritionally rich in omega-3 fatty acids. Each 100 g of mackerel tuna comprises 69,40% water, 25,00% protein, 1,50% fat, and 0,03% carbohydrates, along with a variety of minerals such as calcium, sodium, phosphorus, iron, and vitamin A (retinol), and B (thiamin, riboflavin, and niacin). Mackerel tuna is a rich source of nutrients and fatty acids. Polyunsaturated fatty acids (PUFAs), which are abundant in fish, play an important physiological role in the growth and development of the fetus during pregnancy (Maulu et al., 2021). The nutritional characteristics, texture, and quality of its white and dark muscles make this species a highly suitable raw material in the food industry. Dark muscle is particularly nutritionally valuable, with its fatty acid content predominantly in the form of PUFAs (Hafiludin, 2011).

Based on the above information, previous studies have developed nutrient-rich, crunchy, and savory-intervention food products in the form of mackerel tuna made primarily from mackerel tuna meat. Mackerel tuna is processed as a supplementary food for pregnant women and can be consumed as a side dish with rice or as a daily snack. Therefore, this study aimed to evaluate the macronutrient, fatty acid, and microbiological aspects of the mackerel tuna. The findings of this study are expected to contribute to innovation in supplementary food products for pregnant women, helping address nutritional needs, and combating stunting in Indonesia.

Methods

In previous research, tuna fish products were developed using three formulas: Formula 1, Formula 2, and Formula 3. All developed formulas have the same raw materials, with a difference in the addition of tapioca flour: formula 1 with the addition of 5% tapioca flour, formula 2 with the addition of 15% tapioca flour, and formula 3 with the addition of 25% tapioca flour. The developed products are shown in the following figure.



Formula 1
(5% tapioca flour)



Formula 2
(15% tapioca flour)



Formula 3
(25% tapioca flour)

Figure 1. Three types of mackerel tuna crackers formula

This study was conducted in the laboratory testing was carried out to evaluate the macronutrient content, fatty acid composition, and microbiological safety of the products. These analyses were performed at the Integrated Laboratory of IPB University. Protein levels were determined using the Kjeldahl method of AOAC 2019 (AOAC, 2019). Fat content was determined using the Soxhlet method of

AOAC 2019 (AOAC 2019). Carbohydrate content analysis was performed according to Nielsen 2017 (Nielsen and Nielsen 2017). Fatty acid analysis was performed according to AOAC (2012). Microbiological testing of mackerel tuna crackers included total bacterial count, *Escherichia coli*, and *Salmonella* sp., using modified methods developed (Anang et al., 2018).

To estimate the total bacterial count, first, serial dilutions of 10^{-1} to 10^{-5} were prepared by diluting the mackerel tuna bar sample in distilled water. A 0.1 mL aliquot from dilutions 10^{-3} to 10^{-5} was injected into Petri dishes containing nutrient Agar media. The cells were incubated at 37°C for 24 h. Bacterial colonies were estimated using a colony counter and documented as the total viable count. For *E. coli* testing, a drop of Kovacs reagent was poured into each test tube and the presence of *E. coli* was indicated by red ring formation, indicating the presence of indole. For *Salmonella* sp. testing, a pre-enrichment step with peptone water was followed by enrichment in Rappaport-Vassiliadis (RV) broth. Subsequently, 0.1 mL of the aliquot were inoculated onto Xylose Lysine Deoxycholate (XLD) agar. Suspected *Salmonella* colonies were colorless with a black center because to hydrogen sulfide (H_2S) production.

Result and Discussion

Macro Nutrient Content

Table 1. Macro nutrient content in 100 g of mackerel tuna product

Parameter	Type of Formulas			IOM (2005)	AKG (2019)
	F1	F2	F2		
Water	3,24	3,84	4,32	-	-
Ash	4,36	3,83	3,63	-	-
Protein	39,71	36,92	31,55	73	90
Fat	26,64	21,89	18,34	-	67,3
Carbohydrate	26,05	33,51	42,16	175	400
Fiber	6,39	6,94	6,97	28	34

IOM: Institute of Medicine

AKG: Angka Kecukupan Gizi (Indonesian RDA)

The macronutrient content data in Table 1 show that the protein content was highest in formula 1 (39,71%), while the lowest was in formula 3 (31,55%). The fat content was highest in formula 1 (26,64%) and lowest in formula 3

(18,34%). The carbohydrate content was highest in formula 3 (42,16%) and lowest in formula 1 (26,05%). It can be concluded that the best macronutrient content was in formula 1. The protein content in formula 1 meets 54% of the

protein requirement of pregnant women according to the IOM 2005 recommendation and can meet 44,12% protein requirement of pregnant women according to AKG 2019 (Institute of Medicine (IOM) 2005; Kementerian Kesehatan Republik Indonesia 2019). According to AKG 2019 (Kementerian Kesehatan Republik Indonesia 2019), the fat content in formula 1 is 39,58% of pregnant women. Protein and fat are important nutrients that must be met during pregnancy, and fetal growth and development are completely dependent on protein and fat in the mother's body. Fetal growth and development during pregnancy depends on a continuous supply of nutrients via the placenta (Manta-Vogli et al., 2020). Moreover, both nutrients have a strong correlation with fetal development and nutritional status of birth output (Gala et al., 2016). The results of biochemical studies emphasize that blood lipid concentrations in pregnant women during the late pregnancy period are related to anthropometry of the newborn. High serum lipid levels during this period are imperative for optimal fetal growth (Geraghty et al., 2016). Protein is the nutritional content relied upon in this product for the nutritional status of pregnant women and fetuses. Protein is a fundamental substance that mothers need during pregnancy and is necessary for fetal growth in the mother's womb. This is in line with other studies that concluded that fulfilling protein needs during pregnancy is a major concern because it is crucial in regulating tissue formation and supporting fetal growth, especially in the third trimester. Proteins from fish have the advantage of being easily digested with high biological value and are vital for body growth and development, maintenance, and repair of damaged body tissues, as well as for the generation of hormones and enzymes required for various metabolic processes (Tacon et al., 2020). The increase in protein needs during pregnancy must be met for the development and growth of the fetus and placenta (Marangoni et al., 2016).

Fatty Acid Content

A good nutritional status for fatty acids (FA) during pregnancy is important for the mother's health to meet the needs of fetal growth and development (Aparicio et al., 2021). The

following table shows the fatty acid content of mackerel tuna crackers.

Table 2. Fatty acid content of 100 g mackerel tuna crackers product (%)

Nutritional Value	Formulation		
	F1	F2	F3
Butiric Acid, C4:0	0,04	0,08	0,06
Lauric Acid, C12:0	0,1	0,16	0,2
Myristic Acid, C14:0	0,4	0,63	0,76
Palmitic Acid, C16:0	16,64	27,46	30,75
Palmitoleic Acid, C16:1	0,05	0,11	0,31
Heptadecanoic Acid, C17:0	0,18	0,46	0
Stearic Acid, C18:0	2,01	3,44	3,45
Elaidic Acid, C18:1n9t	0,18	0,24	0,21
Oleic Acid, C18:1n9c	24,23	38,97	43,19
Linolelaidic Acid, C18:2n9t	0,44	0,68	0,66
Linoleic Acid, C18:2n6c	5,58	9,4	10,68
y-Linoleic Acid, C18:3n3	0,16	0,24	0,29
Linoleic Acid, C18:3n3	0,06	0,07	0,08
Docosahexaenoic Acid, C22:6n3	0,36	0,94	0,62
SFA	19,05	31,53	34,96
PUFA	6,16	10,65	11,67
MUFA	24,46	39,32	43,71

Based on the data in Table 2, the Total PUFA content of the product was 6,16%. Unsaturated fatty acids can be divided into monounsaturated fatty acids and polyunsaturated fatty acids according to the number of double bonds. Polyunsaturated fatty acids (PUFAs) are a class of fatty acids that include n-6 and n-3 fatty acids (Liu et al., 2023). Emerging evidence suggests that omega (n)-3 PUFA and their metabolites improve maternal and neonatal health outcomes by modifying gestation length and reducing the recurrence of preterm delivery. N-3 PUFA have been associated with prolonged gestation and increased birth dimensions, such as birth weight and head circumference.

Supplementation of maternal diet with n-3 PUFA has been shown to have a positive effect on fetal brain development and reduce the recurrence of PTB, especially in women with a history of preterm or low baseline n-3 PUFA intake (high-risk population) (Akerlele dan Cheema 2016). Among the known fatty acids, omega-3 (n-3) and omega-6 (n-6) PUFAs are the most essential because of their biological functions such as lowering oxidative stress, affecting inflammatory cascades, and providing neuroprotection and cardiovascular protection. These two types of fatty

acids are essential and include cis-type PUFAs, specifically omega-3 fatty acids (such as α -linolenic acid, eicosatetraenoic acid/EPA, and docosahexaenoic acid/DHA), and omega-6 fatty acids (such as linoleic acid). The total omega-3 fatty acid content was the highest in formula 2 (1,18%). The omega-6 fatty acid content in the samples was highest for formula 3 (10,78%). These values fall within the recommended range 1–5 for the omega-3 to omega-6 fatty acid ratio per day, as suggested by food experts to prevent diseases associated with a deficiency in essential fatty acid intake (EFSA Panel on Dietetic Products and Allergies (EFSA Panel on Dietetic Products and Allergies (NDA) 2010). Furthermore, among the omega-3 fatty acids, eicosatetraenoic acid (EPA) and docosahexaenoic acid (DHA) are particularly important for the human body, and approximately 0,2–2,0 g/day is suggested by most health organizations (Desai *et al.* 2018). The total monounsaturated fatty acids (MUFA) in the product were 24,46%. The most abundant MUFA were oleic acid and palmitic acid. Oleic Acid was the highest in formula 3 (43,19%).

The total saturated fatty acid (SFA) of the product was 19,05%. SFA may play a key role in supporting fetal membrane growth, as the SFA biomagnification process could help satisfy AA demands in fetal circulation and DHA in the brain (Ogundipe *et al.*, 2016). This type of fatty acid is not recommended for excessive consumption, but in moderate amounts, it is beneficial for pregnant women, as they need fatty acids for both their own and their babies' health during pregnancy. Palmitic Acid, was the most abundant in formula 3 (30,75%).

Bacterial Count

All samples were evaluated using several tests, including total bacterial count (TPC), Salmonella spp., and Escherichia coli. A nonselective medium was used for the total bacterial count, and the results showed that none of the processed samples surpassed the maximum limit allowed for microbial contamination of food products introduced by the Indonesian National Standards or SNI (Badan Standard Nasional 2009).

Table 3 shows that the total bacterial count in the product was below the threshold limit. The counts of *E. coli* and *Salmonella* in the products were below the threshold. Overall, it can be concluded that the pathogenic bacteria present in all three product formulas are below the food safety limits. Furthermore, after detecting *Salmonella* sp. and *E. coli* in the mackerel tuna cake using specific media, no

bacterial growth (negative) was observed. Hence, the outcomes comply with the microbial contamination requirements for dried fish products (Badan Standard Nasional, 2009). The mackerel tuna cake product used in this study was in accordance with the SNI 7988:2009.

Table 3. Microbiology aspect of mackerel tuna crackers product

Bacterial Test	F1	F2	F3	SNI*
TPC	<10	<10	<10	<1 × 10 ⁵
<i>Salmonella</i> Sp.	<3	<3	<3	<3
<i>E. coli</i>	Negative	Negative	Negative	Negative

*SNI 7988:2009: maximum permissible limits of microbial contamination in food products.

Microbiological tests are important for ensuring the safety of tuna fish plate products in pregnant women. Pregnant women are a vulnerable group, particularly if they experience malnutrition and are at risk of infection when consuming food contaminated with pathogenic bacteria. Ensuring product safety from pathogenic bacteria that could harm pregnant women helps reduce the threat of maternal infections and supports the well-being of the unborn baby.

Conclusion

The results showed that the protein content of the product was highest in formula 1 (39,71%), meeting 54% of the protein requirement of pregnant women according to the IOM 2005 recommendation and 44,12% protein requirement of pregnant women according to AKG 2019. The total omega-3 content was highest in formula 2 (1,18%), and the total omega-6 content was highest in formula 3 (10,78%). The results of the microbiological tests showed that all products were safe according to SNI 7988:2009. Further studies on the efficacy of mackerel tuna crackers as a nutritional intervention in pregnant women are urgently required.

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