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Analysis of Protein Content, Iron Content, and Acceptability of Crackers Substituted with Edamame (*Glycine max L. Merrill*) Flour and Mackerel Tuna (*Euthynnus affinis*) Protein Hydrolysate

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ABSTRACT

Crackers are a snack often favored by toddlers. Commercially available crackers predominantly use wheat flour, which is high in carbohydrates but low in nutritional value, particularly in protein content. Therefore, innovative formulations are needed to enrich the nutrition, notably protein and iron, by substituting edamame flour and mackerel tuna protein hydrolysate into crackers to help address stunting. This study aims to analyze the protein content, iron content, and acceptability of crackers made with a substitution of edamame flour and tuna protein hydrolysate. This research was conducted at the University of Jember from February to April 2024 using a completely randomized design at four levels. Protein analysis used the Kjeldahl method, and iron content was examined by atomic absorption spectrometry. The acceptability was tested using the hedonic scale test on 30 semi-trained panelists. The results were analyzed using SPSSv24. Results showed statistically significant differences in protein content, iron content, color acceptability, aroma, and taste. The highest protein content is found in A3, while the highest iron content is found in A1. The best formulation, determined by the exponential comparison method, was treatment A2. In conclusion, substitution with edamame flour and tuna protein hydrolysate in all treatment variations significantly affected protein content, iron content, and acceptability in terms of color, aroma, and taste; however, it did not substantially impact texture or overall acceptability. Substitution of edamame flour and mackerel tuna protein hydrolysate in crackers can increase protein content and therefore could be an effective measure in preventing stunting.

Keywords : Stunting; edamame flour; mackerel tuna; fish protein hydrolysate; crackers.

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INTRODUCTION

Stunting is characterized by impaired growth that results from prolonged nutritional inadequacy (1). Globally, 23.2% or 150.2 million children under 5 years of age were too short for their age (stunting) in 2024 (2). According to the 2022 SSGI, the prevalence of stunting among Indonesian toddlers reached 21.6%, with Jember Regency recording the highest prevalence in East Java at 34.9% (3). One key determinant of stunting is low intake of protein and iron. Protein is a major nutrient essential in child development, while iron is necessary for cognitive and physical function; deficiencies can lead to impaired growth, including stunting (4).

Jember Regency produces edamame (*Glycine max (L.) Merrill*) as a leading agricultural product, which is high in protein, iron, calcium, and phosphorus. The high protein and iron content of edamame can be utilized to prevent stunting. In this research, edamame is processed into flour to further support nutritional value, extend storage stability, and facilitate its use in food products such as crackers. Edamame beans contain 10.59 g/100 g of protein and 2.12 mg/100 g of iron. Processing edamame into flour increases its protein (41.3 g/100 g) and iron (9.53 mg/100 g) content compared to the raw bean (5). This flour improves nutritional value and the practicality of product formulation, such as in crackers—a convenient, long-lasting snack widely favored yet typically offering limited protein content (6). However, commercially available crackers contain very low protein levels, providing only about 5% of the daily protein requirement per serving. To enhance the nutritional value, particularly protein and iron content, wheat flour was substituted with edamame flour (7). Cracker biscuits, commonly referred to as crackers, are classified as baked cereal-based snack products distinguished by their thin geometry, low moisture composition, and characteristically brittle texture. The consumption dynamics of these products are governed by multifactorial determinants, including market availability, economic feasibility, organoleptic attributes, and sociocultural paradigms. Despite such variability, crackers maintain substantial global popularity attributable to their convenience in consumption, prolonged shelf stability, and functional versatility within diverse nutritional and culinary applications (8).

In addition to edamame flour substitution, mackerel tuna was incorporated into the crackers in the form of fish protein hydrolysate to increase protein content and provide a complete profile of essential amino acids necessary for growth. Mackerel tuna fish is an important fishery commodity in Jember, with a production level of 627.91 tons (9). Mackerel tuna is a source of major nutrients, including protein (13.7 g/100 g) and iron (1.6 mg/100 g), fat (0.2 g), vitamins A and B, and minerals such as calcium, phosphorus, iron, and sodium (10,11). In this study, mackerel tuna was processed into fish protein hydrolysate by hydrolyzing fish protein into shorter peptides or amino acids through chemical or enzymatic means. The resulting powder is easier to substitute, more readily absorbed by the body, and improves the quality of the final product (12). Compared to fish flour, protein hydrolysate was chosen because it is less fishy in aroma and has higher solubility, enhancing its absorption (13,14).

Crackers made with edamame flour and tuna protein hydrolysate are expected to serve as supplementary foods rich in protein and iron, suitable for stunting prevention in toddlers. To date, no studies have reported the incorporation of hydrolyzed mackerel tuna protein as a substitute ingredient in crackers formulations, thereby motivating researchers to explore its feasibility and potential functional properties in this context.

METHOD

This research has received ethical approval from the Ethical Committee of Medical Research Faculty of Dentistry, University of Jember, with Number 2435/UN25.8/KEPK/DL/2024. This quantitative research was conducted from February to April 2024 using a completely randomized design at four levels with three replications. The production of edamame flour and mackerel tuna protein hydrolysate took place at the Faculty of Agricultural Technology, University of Jember. Protein and iron analysis were carried out at the Food Technology Laboratory, State Polytechnic of Jember. Acceptability testing was conducted at Arjasa State Primary School 01, Jember Regency.

The main ingredients used in this study included edamame beans, tuna fish, wheat flour, milk, vegetable oil, sugar, yeast, baking powder, and water. The chemical reagents were papain enzyme, distilled water, potassium sulfate (K_2SO_4), copper sulfate ($CuSO_4$), sulfuric acid (H_2SO_4), sodium hydroxide (NaOH), boric acid (H_3BO_3), hydrochloric acid (HCl), and nitric acid (HNO_3). The instruments utilized consisted of an 80 mesh sieve, water bath, centrifuge, and freeze dryer. Analytical processes required additional apparatus such as an analytical balance, Erlenmeyer flask, burette, pipette, ashing dish, and furnace.

Preparation of edamame flour was conducted according to the Siregar method in 2023, involving peeling, soaking in water at a ratio of 3:1 for eight hours, boiling for 20 minutes at 100°C, drying in an oven at 60°C for twenty-four hours, and milling using an 80 mesh sieve (15). Edamame possesses a firm outer pericarp that must be removed before subsequent processing steps. Pre-treatment through soaking, blanching, and drying effectively inactivates lipoxygenase activity, thereby minimizing the formation of volatile compounds responsible for undesirable beany odors. The treated seeds are subsequently subjected to milling to produce edamame flour, which demonstrates enhanced storage stability and reduced susceptibility to oxidative rancidity and microbial deterioration, making it a viable ingredient for incorporation into various formulated food products. Preparation of mackerel tuna protein hydrolysate powder followed the Witono method in 2020, which consisted of separating the fish meat from bones, weighing, blending with distilled water at a 1:2 ratio, adding 5% papain enzyme, hydrolyzing at 55°C for 3 hours, inactivating the enzyme at 85°C for 20 minutes, cooling to room temperature (27°C), and centrifuging at 3500 rpm and 10°C for 30 minutes (16). Studies indicate that concentrations around 5% papain enzyme ensure efficient protein breakdown while maintaining the desired bioactive properties and functional quality of the hydrolysate, making it suitable for food and nutraceutical applications (17). In the manufacture of fish protein hydrolysate, the hydrolysis stage conducted at 55°C for 3 hours serves to optimize the activity of proteolytic enzymes used to break down fish proteins into smaller peptides and amino acids. This temperature is selected because many enzymes, such as alcalase, papain, or flavourzyme, exhibit their optimal catalytic activity around 50–60°C, which allows efficient cleavage of peptide bonds without causing enzyme denaturation or excessive protein damage. The 3-hour duration ensures sufficient time for hydrolysis to reach a desirable degree, improving the yield of bioactive peptides that contribute to functional properties (18). The resulting supernatant is then frozen and freeze-dried into a powder. Production of crackers employed a modified Wijaya method, including mixing yeast with warm water until foamy to ensure the yeast is active, combining all ingredients, kneading the dough to develop texture, resting the dough for 30 minutes to allow gluten relaxation, which reduces dough elasticity and facilitates easier handling during rolling and shaping. This resting phase also enables thorough hydration and uniform distribution of moisture throughout the dough matrix, enhancing the structural integrity and texture of the final product. Additionally, it promotes optimal dough development, which subsequently improves the crispness and sensory attributes of the baked crackers. The next step is rolling the dough, shaping, and baking in an oven at 170°C for fifteen minutes (19).

Protein content analysis in crackers using the Kjeldahl method according to SNI 01-2981-1992 standards. This method consists of three main steps. The first stage is the digestion of the sample, mixed with K_2SO_4 (7g) and $CuSO_4$ (0.8g), and heated until the solution turns a clear blue-green. After cooling, the process proceeds to the distillation stage by adding a NaOH solution (50 ml). The mixture is heated to liberate ammonia gas, which is then passed into a H_3BO_3 solution (30 ml). The final step involves titrating the captured ammonia with hydrochloric acid (HCl) until a pink color change indicates the titration endpoint.

Iron content analysis in crackers was based on the Apriyantono method, starting with placing the sample in an ashing dish, ashing in a furnace at 450°C for one hour, cooling, adding 1.0 ml HNO_3, destructing on a hot plate, returning to the furnace, adding 1.0 ml HCl, 2.0 ml deionized water, 1 ml vortex hydroxide, and stirring. After 30 minutes, absorbance was read at 533 nm with spectrophotometer model (20).

The ratios and substitutions of ingredients for cracker production were determined from Handayani's research (21). Table 1 presents the proportions of wheat flour, edamame flour, and tuna protein hydrolysate used in the cracker formulations. To date, there is no documented research investigating the substitution of fish protein hydrolysate (FPH) in cracker formulations. Consequently, preliminary substitution levels were established at 2.5%, 5%, and 7.5% to evaluate their effects on product quality attributes. In a related study involving biscuit formulations, the incorporation of FPH at 5% yielded the most favorable shelf-life stability compared to higher substitution levels of 10% and 15% (22).

Table 1. Proportion of Crackers with Edamame Flour and Mackerel Tuna Protein Hydrolysate Substitution per 100 g

Treatment	Mackerel Tuna Protein Hydrolysate	Edamame Flour	Wheat Flour
A0	0%	0%	100%
A1	2.5%	22.5%	75%
A2	5%	20%	75%
A3	7.5%	17.5%	75%

The acceptability test for these crackers in this study utilized a hedonic scale test assessing five parameters: color, aroma, taste, texture, and overall acceptance. There are four hedonic scale categories, including "like very much," "like," "dislike," and "dislike very much." The panelists consisted of 30 untrained elementary school children in the fifth grade, aged 11 to 12 years. The data obtained from the hedonic scale test were analyzed using the SPSS software, first with the Mann-Whitney test, followed by the Wilcoxon test, as the data were ordinal. Subsequently, the datasets of protein and iron levels were assessed for normality. Variables conforming to a normal distribution were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference (HSD) post hoc test. Conversely, variables deviating from normality were examined using the Kruskal–Wallis test, with pairwise comparisons performed using the Mann–Whitney U post hoc analysis.

RESULTS

Protein and Iron Levels

Based on laboratory analysis, the protein and iron levels of crackers substituted with edamame flour and mackerel tuna protein hydrolysate were as follows.

Table 2. Protein and Iron Level of *Crackers* (100 g)

Parameter	A_0	A_1	A_2	A_3	*p*
Protein (g)	8.74 ± 0.09^{b}	11.78 ± 0.16^{ab}	11.96 ± 0.03^{ab}	12.19 ± 0.04^{b}	0.020*
Iron (mg)	0.67 ± 0.01^{b}	1.81 ± 0.01^{b}	1.5 ± 0.03^{b}	1.37 ± 0.01^{b}	0.021*

*A significant difference was observed at a test level of p <0.05
^{ab}Mann–Whitney Test: a = no significant difference; b = significant difference

Table 2 shows that protein content increases with increasing amounts of fish protein hydrolysate (highest in A3). This indicates that mackerel tuna protein hydrolysate can increase the protein content of crackers. Conversely, iron levels increased with increasing amounts of edamame flour (highest in A1). This increase is attributed to edamame flour being the primary source of iron in this product formulation.

Acceptability Test

Based on the hedonic scale test, the crackers were evaluated in terms of color, taste, aroma, texture, and overall acceptance.

Table 3. Acceptability Analysis of *Crackers*

Parameter	A_0	A_1	A_2	A_3	*p*
Color	3.20 ± 0.41[a]	3.37 ± 0.56[a]	3.33 ± 0.60[a]	3.03 ± 0.49[ab]	0.03*
Aroma	2.83 ± 0.53[a]	2.63 ± 0.56[a]	3.23 ± 0.62[b]	3.27 ± 0.52[b]	0.00*
Taste	3.33 ± 0.60[a]	2.93 ± 0.64[b]	3.43 ± 0.62[a]	3.40 ± 0.92[a]	0.02*
Texture	3.20 ± 0.71	3.27 ± 0.74	3.27 ± 0.69	3.27 ± 0.45	0.89
Overall	3.23 ± 0.57	3.00 ± 0.64	3.40 ± 0.49	3.37 ± 0.55	0.07

*A significant difference was observed at a test level of $p < 0.05$
[ab]Wilcoxon Test: a = no significant difference; b = significant difference
Scale: 1 = strongly dislike; 2 = dislike; 3 = like; 4 = strongly like

Based on the data presented in Table 3, statistical analysis indicated significant differences in the color, aroma, and taste parameters, whereas no significant differences were observed in the texture and overall appearance parameters. Color plays a critical role in food acceptance as it stimulates visual appeal (23). The hedonic test results showed that treatment A3 received the lowest sensory score for color due to its dark cream appearance, while A1 was the most preferred, exhibiting a light greenish cream tone. The Friedman test indicated a significant difference in color resulting from varying levels of edamame flour and mackerel tuna protein hydrolysate substitution.

Based on the aroma parameter, the A3 treatment demonstrated the highest level of sensory preference among the evaluated formulations. characterized by a distinctive savory scent. Treatment A1 received the lowest preference score, associated with a bean-like smell, as aroma characteristics can influence perceived flavor even before consumption. Regarding taste, the most preferred crackers were from treatment A2 with an average score of 3.43, followed by A3, A0, and A1. The Friedman test for taste revealed a significant difference among the four samples.

Based on the texture parameter, treatments A1, A2, and A3 yielded the highest average scores (3.27), while A0 (100% wheat flour) received the lowest score due to its harder texture compared to the other formulations. Overall acceptance, reflecting panelists' preferences for color, taste, aroma, and texture, showed that treatment A2 had the highest mean score (3.34). In contrast, treatment A1 had the lowest overall preference score (3.00). The Friedman test results indicated no significant difference in overall acceptance among the treatments containing various ratios of edamame flour and mackerel tuna protein hydrolysate substitution.

Recommendation for Crackers Consumption Based on Optimal Formulation

From the calculation using the Exponential Comparison Method, the optimal formulation was determined as shown in Table 4, with a scoring scale ranging from 1 (lowest) to 4 (highest). The best treatment was identified as formulation A2 (20% of edamame flour; 5% of mackerel tuna protein hydrolysate).

According to the Recommended Dietary Allowance (RDA), the daily protein requirement for toddlers aged 1–3 years is 20 g, while for those aged 4–5 years it is 25 g. The daily iron requirement is 7 mg for toddlers aged 1–3 years and 10 mg for those aged 4–5 years. Additional food consumption is typically provided twice daily, with each serving representing 10% of the daily requirement. Therefore, the protein requirement from supplementary foods is 4 g/day for toddlers aged 1–3 years and 5 g/day for those aged 4–5 years.

Table 4. Determination of The Best Formulation

Parameter	Weighting of Values	Alternative Component Scores							
		A_0		A_1		A_2		A_3	
		Scale	Score	Scale	Score	Scale	Score	Scale	Score
Protein level	40%	1	0.4	2	0.8	3	1.2	4	1.6
Iron level	15%	1	0.15	4	0.6	3	0.45	2	0.3
Color	10%	2	0.2	4	0.4	3	0.3	1	0.1
Aroma	10%	2	0.2	1	0.1	3	0.3	4	0.4
Taste	10%	2	0.2	1	0.1	4	0.4	3	0.3
Texture	8%	1	0.08	2	0.16	2	0.16	2	0.16
Overall	7%	2	0.14	1	0.07	4	0.28	3	0.21
Total Score	100%		1.37		2.23		3.09		3.07
Ranking			4		3		1		2

Similarly, the iron requirement from supplementary foods is 1.4 mg/day for toddlers aged 1–3 years and 2 mg/day for those aged 4–5 years. Each cracker contains 0.299 g of protein and 0.0373 mg of iron. Based on the optimal formulation (A2), the recommended daily consumption is 14 crackers for toddlers aged 1–3 years, and 16 crackers for those aged 4–5 years.

DISCUSSION

Protein Content

The highest protein content in crackers substituted with edamame flour and mackerel tuna protein hydrolysate was found in treatment A3 (7.5% of mackerel tuna protein hydrolysate; 17.5% of edamame flour). The lowest average protein content was observed in treatment A0, which had no substitution of either edamame flour or mackerel tuna protein hydrolysate. The findings indicate that protein content increased with higher proportions of mackerel tuna protein hydrolysate. This result corresponds to the fact that mackerel tuna protein hydrolysate contains a high protein concentration of 25.68 g per 100 g — approximately double that of mackerel tuna meat (13.7 g per 100 g). Furthermore, fish protein hydrolysates are known for their heat stability; thus, the protein content of mackerel tuna protein hydrolysate remains relatively unchanged during cracker baking (24). This finding is consistent with the results of Asare, who reported that increasing sardine protein hydrolysate content in biscuits proportionally increased total protein levels (12). In contrast, plant-based protein from edamame flour is more sensitive to heat than animal protein. During food extraction and processing, high temperatures can accelerate protein denaturation and decrease the thermal stability of plant-based proteins, leading to a reduction in protein content after heating compared to before thermal processing (25). This indicates that the hydrolysate not only increases protein content but also enhances the protein quality due to its peptide composition.

Iron Content

The highest iron content in crackers substituted with edamame flour and mackerel tuna protein hydrolysate was recorded in treatment A1 (22.5% of edamame flour; 2.5% of mackerel tuna protein hydrolysate). The lowest iron content was observed in treatment A0, which had no substitution. The study found that iron levels increased with greater substitution of edamame flour, primarily because edamame flour serves as the main source of iron in these crackers. One hundred grams of edamame flour contains 9.3 mg of iron, significantly higher than whole edamame beans (2.12 mg) (5). Variations in edamame flour (0%, 17.5%, 20%, 22.5%) and mackerel tuna protein hydrolysate (0%, 2.5%, 5%, 7.5%) produced significant differences in the iron content of the resulting crackers.

Acceptability Test

Color evaluation revealed that increased substitution with edamame flour generated greener crackers due to the presence of chlorophyll in edamame. This aligns with the findings of Wibowo, who reported that higher edamame flour substitution yielded greener cookies (26). Conversely, greater substitution with mackerel tuna protein hydrolysate resulted in darker cracker coloration, a result of Maillard reactions between proteins and reducing sugars during baking (16). These reactions darken the product color, consistent with Handayani's research on the addition of catfish protein hydrolysate to cassava crackers, which also intensified the color (21) Panelists most preferred the color of A1 crackers, described as light greenish cream, corresponding to lower mackerel tuna protein hydrolysate substitution, which prevented excessive darkening relative to A2 and A3.

Crackers with higher edamame flour substitution exhibited a stronger beany aroma caused by volatile compounds such as n-hexanal, 1-hexanol, 2-hexanal, 3-hexene-1-ol, and phenylethyl alcohol. This observation is consistent with Wibowo's findings that higher edamame flour substitution intensifies the beany scent in cookies (27). In contrast, higher mackerel tuna protein hydrolysate substitution produced a savory aroma due to increased concentrations of glutamic and aspartic acids (24). The stronger the edamame aroma, the lower the aroma acceptability. Thus, panelists preferred crackers from treatment A3, characterized by the highest protein hydrolysate substitution (7.5%) and a distinct fish-like savory note. This is consistent with Mervina's findings, which showed that increasing catfish hydrolysate and soy protein isolate substitution elevated aroma acceptance levels in biscuits (28).

Regarding taste attributes, the hedonic evaluation demonstrated a progressive increase in preference scores from treatment A1 to A3. This trend was attributed to the increasing intensity of bitterness associated with higher proportions of edamame flour. The incorporation of edamame flour contributed to a beany and slightly bitter flavor profile, primarily linked to the presence of unsaturated fatty acids and isoflavone compounds (27). In contrast, higher substitution levels of mackerel tuna protein hydrolysate imparted a more pronounced savory and umami flavor, attributed to the presence of oligopeptides enriched in glutamic and aspartic acids that activate umami taste receptors (24). Although edamame flour can impart bitterness, the presence of mackerel tuna protein hydrolysate balanced this flavor, resulting in a more neutral taste (27). Similar findings were reported by Handayani, who noted that adding catfish hydrolysate enhanced the savory flavor and taste preference of cassava crackers (21). This resulted in treatment A1 having the lowest preference level, while A3 had the highest preference level.

Texture evaluation indicated that substitution with edamame flour and mackerel tuna protein hydrolysate resulted in softer crackers (A1, A2, and A3) compared to A0 (control), which had a harder texture due to the absence of substitution and the high gluten content of wheat flour. Edamame flour, being low in gluten, reduces starch gelatinization, resulting in a less rigid and more brittle texture. This observation supports the findings of Ni Wayan, who showed that higher gluten-free green bean flour content increased the brittleness of snack bars (29). Moreover, children generally prefer foods with softer textures due to their oral physiological adaptability (30). In cracker formulations A1, A2, and A3, variations in edamame flour concentration did not result in statistically significant differences, indicating that panelists did not perceive notable distinctions among the treatments.

The overall acceptability parameter represents an integrated measure of sensory quality encompassing color, aroma, taste, and texture attributes. Although statistical analysis revealed no significant differences among treatments, formulation A2 obtained the highest overall acceptability score from panelists. This treatment exhibited the most balanced sensory profile, indicating its potential as the optimal formulation.

Recommended Cracker Consumption Based on Optimal Formula

The Exponential Comparison Method identified A2 (20% edamame flour and 5% mackerel tuna protein hydrolysate) as the optimal formulation. The basic cracker recipe referenced from Wijaya's formulation was modified by substituting part of the wheat flour with edamame flour and protein hydrolysate according to prior studies (19). For a 100 g recipe, the formulation included 37.5 g wheat flour, 10 g edamame flour, and 2.5 g mackerel tuna protein hydrolysate, along with other complementary ingredients.

According to the Recommended Dietary Allowances (RDA), toddlers aged 1–3 years require 20 g of protein and 7 mg of iron daily, while those aged 4–6 years require 25 g of protein and 10 mg of iron (31). Supplementary foods are typically consumed twice daily, comprising 10% of total daily intake per serving, or 20% overall. A cracker weighs approximately 2.5 grams. Based on protein adequacy levels from A2, toddlers aged 1–3 years are recommended to consume 14 crackers daily, satisfying 21% of their daily protein and 7.4% of their daily iron. Toddlers aged 4–5 years should consume 16 crackers daily, fulfilling 19% of their protein requirement and 6% of their iron requirement. These findings comply with the Indonesian National Standard (SNI 2973:2011), which specifies that biscuits must contain at least 5% protein (32). When consumed at the recommended intake level, it can contribute significantly to the enhancement of daily dietary protein and iron intake derived from snack consumption and is anticipated to play a supportive role in stunting prevention efforts.

CONCLUSIONS AND RECOMMENDATIONS

This study demonstrates that the incorporation of edamame flour and mackerel tuna protein hydrolysate significantly enhances protein and iron contents without adversely affecting sensory quality. The selected formulation, A2, exhibited an optimal balance among protein concentration, iron content, and sensory acceptability. Based on the Recommended Dietary The findings of this study are expected to serve as a reference for food industry practitioners, offering an innovative approach for developing diverse supplementary feeding products for the Ministry of Health of the Republic of Indonesia. Furthermore, future researchers are encouraged to investigate the potential benefits of crackers with edamame flour and mackerel tuna protein hydrolysate substitutions as snacks for toddlers to support stunting prevention, while reconsidering the optimal proportion of edamame flour used and analyze the bioavailability of protein and iron in the final product

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