Jurnal Gizi dan Dietetik Indonesia
(Indonesian Journal of Nutrition and Dietetics)
Vol 13, Issue 1, 2025: 1-9

Available online at: https://ejournal.almaata.ac.id/index.php/IJND
p-ISSN 2303-3045 e-ISSN 2503-183X
DOI: https://dx.doi.org/10.21927/ijnd.2025.13(1).1-9

The formulation of edamame flour and tuna fish protein hydrolyzate biscuit as an alternative supplementary feeding for stunting

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ABSTRAK

Latar Belakang: *Stunting keadaan kurang gizi paling umum yang terjadi di Indonesia dan masih menjadi permasalahan yang harus ditangani saat ini. Salah satu upaya untuk memperbaiki kondisi stunting pada balita yaitu dengan memenuhi kebutuhan protein melalui PMT yang tinggi protein. Biskuit dapat menjadi salah satu media dalam perbaikan zat gizi apabila ditambahkan dengan bahan tertentu. Pangan lokal yang dapat ditambahkan dalam bahan pembuatan biskuit adalah kacang kedelai edamame yang dioah menjadi tepung edamame dan ikan tuna yang dijadikan hidrolisat protein ikan (HPI).*
Tujuan: *Tujuan dari penelitian ini adalah untuk mengetahui formulasi terbaik dari biskuit tepung edamame dengan tambahan HPI tuna untuk memperbaiki kondisi stunting pada balita.*
Metode: *Penelitian ini menggunakan Rancangan Acak Lengkap (RAL) faktor tunggal dengan tiga sampel, yaitu perbandingan formulasi tepung edamame dengan HPI tuna dengan 3 taraf (F1 30:10, F2 25:15, dan F3 20:20). Penelitian dimulai dengan pembuatan tepung edamame, HPI tuna, dan biskuit tepung edamame dan HPI tuna. Selanjutnya biskuit tepung edamame dan HPI tuna diuji organoleptik yang meliputi kesukaan warna, rasa, aroma, tekstur, dan keseluruhan pada 25 panelis semi terlatih. Formulasi terpilih akan dianalisis kadar protein, lemak, dan karbohidrat.*
Hasil: *Berdasarkan uji organoleptik dan total hasil pembobotan didapatkan bawah formulasi biskuit terpilih adalah F3. Hasil uji Kruskal Wallis menunjukkan bahwa penambahan tepung edamame dan HPI tuna berpengaruh nyata terhadap kesukaan warna ($p<0,05$). Berdasarkan uji laboratorium diketahui jika formula biskuit terpilih (F3) memiliki kandungan gizi per 100 gram: protein 16,81±0,07 gram; lemak 19,66±0,14 gram; karbohidrat 49,05±0,06 gram.*
Kesimpulan: *Penelitian ini menunjukkan bahwa formulasi biskuit terpilih adalah F3 dengan kandungan tepung edamame 20% dan HPI tuna 20%. Kandungan protein dan lemak pada biskuit terpilih (F3) lebih tinggi dibandingan dengan biskuit PMT Pabrikan.*

KATA KUNCI*:* *biskuit; hidrolisat protein ikan; ikan tuna; tepung edamame; stunting*

ABSTRACT

Background: *Stunting is the most common malnutrition condition that occurs in Indonesia and is still a problem that has to be solved today. One of the efforts to improve stunting conditions in toddlers is by fulfilling the protein needs through supplementary feeding which has high protein. Biscuits can be a medium for improving nutrition if they are added with certain ingredients. Local foods that can be added to biscuits are edamame soybeans which are processed into edamame flour and tuna which is made into fish protein hydrolyzate (FPH).*

Objectives: *To find out the best formulation of edamame flour biscuits with the addition of tuna FPH to improve stunting conditions in toddlers.*

Methods: *This research used a single factor Completely Randomized Design (CRD) with three samples, which were a comparison of edamame flour formulation with tuna FPH with 3 levels (F1 30:10, F2 25:15, and F3 20:20). The research began with making edamame flour, FPH tuna, and the biscuits. Furthermore, edamame flour and tuna FPH biscuits were tested organoleptically which included color, taste, aroma, texture, and overall preferences of 25 semi-trained panelists. The selected formulation will be analyzed for protein, fat, and carbohydrate levels.*

Results: *Based on organoleptic tests and the total weighting results obtained the selected biscuit formulation was F3. The results of the Kruskal Wallis test showed that the addition of edamame flour and tuna FPH had a significant effect on color preference ($p<0.05$). Based on laboratory tests, it is known that the selected biscuit formula (F3) has nutritional content per 100 grams: protein 16.81±0.07 g; fat 19.66±0.14 g; carbohydrate 49.05±0.06 g*

Conclusions: *The selected biscuit formulation is F3 with 20% edamame flour content and 20% tuna FPH. The protein, fat, and carbohydrate content of the selected biscuits (F3) is higher than the supplementary feeding manufactured biscuits.*

KEYWORD*:* *biscuit****;*** *edamame flour; fish protein hydrolyzate; stunting; tuna*

Article info:
Article submitted on May 05, 2024
Articles revised on June 06, 2024
Articles received on July 29, 2024

INTRODUCTION

Stunting is the most common malnutrition condition in Indonesia. Stunting is a condition where the children have developmental and growth disorders which are the result of a lack of food intake (1). It is identified by a height-for-age index with a z-score of below -2 SD (2). Stunting can cause various growth and development disorders, including impaired physical growth, impaired brain development, and intelligence, and cause children to be vulnerable to various diseases. This makes stunting a major threat to the quality of human resources in Indonesia (1). In 2022, the prevalence of stunting in Indonesia will be 21.6% (3). This figure is still far from the target (14%) of Indonesia's National Medium-Term Development Planning in 2024 (4). Based on The Indonesian Nutritional Status Survey data (2022), the prevalence of stunting in East Java is 19.2% with the highest prevalence of stunting in the Jember Regency which reaches 34.9% (5). The high rate of stunting in Indonesia, especially in Jember Regency, is still a top priority that must be immediately resolved by various parties including the government, health workers, and the community because stunting can hurt Indonesia's future.

One effort to improve stunting conditions in toddlers is by meeting protein needs through supplementary feeding which is high in protein based on local food. The Jember Regency is one of the largest producers of edamame beans in East Java, however, the use of edamame beans is still not widely known to the general public so the level of consumption is still not optimal even though it has a high nutritional content (6). In addition to abundant edamame bean production, Jember Regency as a coastal area is also rich in marine products, one of which is tuna. Edamame beans and tuna are local foods with a high protein content. The protein content in 100 g of fresh edamame beans is 12.4 g (7), while the protein content in 100 g of fresh tuna is 28.34 g (8). One of the nutritional improvement media that can be modified is biscuits. Biscuits can be a medium for improving nutrition if certain ingredients are added (9). Edamame beans can be processed into flour as a raw material for cookies, pudding, meatballs, and other food products. In 100 grams, edamame flour has a protein content of 37.41%, which is higher than in its fresh form (10). On the other hand, tuna can be processed into fish protein hydrolyzate (FPH). Fish protein hydrolyzate is a form of dry protein with a protein content exceeding 60%, the result of the breakdown of fish protein through hydrolysis by enzymes, acids, or bases. The addition of FPH to various products is expected to increase protein consumption and nutritional quality (11). The addition of edamame flour and tuna fish protein hydrolyzate to biscuits is expected to be an effective solution in overcoming stunting. Therefore, this study aims to determine the best formulation of edamame flour and tuna fish protein hydrolysate biscuit to improve stunting conditions in toddlers.

MATERIALS AND METHODS

This research used a single factor Completely Randomized Design (CRD) with three samples, that were a comparison of edamame flour formulations with tuna FPH. Each treatment was repeated three times. The formulation of edamame flour and tuna FPH can be seen in **Table 1**. This research was done in three stages, that were making edamame flour, making powdered tuna FPH, and making edamame flour and tuna FPH biscuits. The composition of these biscuits is detailed in **Table 2**. The next step is to test the characterization of the chemical and organoleptic properties of biscuit products formulated with edamame flour and FPH tuna.

Table 1. The Formulation of Edamame Flour and Tuna FPH

Formulation	Wheat flour (%)	Edamame flour (%)	Tuna FPH (%)
F1	60	30	10
F2	60	25	15
F3	60	20	20

This research was done at the Laboratory of Chemical and Biochemical Agricultural Product and Agricultural Product Engineering Laboratory, Faculty of Agricultural Technology, Universitas Jember.The research was conducted using a variety of tools, including digital analytical balances, freeze dryers, mixers, beaker glasses, water bath shakers, food processors, 80 mesh Tyler sieves, centrifuges, pots, stoves, knives, measuring cups, baking trays, baking ovens, bowls, and spoons. In addition, glassware, Soxhlet extraction equipment, Kjeldahl flasks, burettes, ovens, furnaces, desiccators, porcelain cups, iron spatulas, measuring pipettes, measuring flasks, erlenmeyers, glass funnels, and drop pipettes were used for analysis.

Table 2. Composition of Edamame Flour and Tuna FPH Biscuits

Materials	F1	F2	F3
Wheat flour (g)	27	27	27
Edamame flour (g)	13.5	11.25	9
Tuna FPH (g)	4.5	6.75	9
Refined sugar (g)	18	18	18
Skim milk (g)	4.74	4.74	4.74
Vanilla (g)	0.06	0.06	0.06
Baking powder (g)	0.2	0.2	0.2
Margarine (g)	14	14	14
Egg yolk (g)	11	11	11
Water (g)	7	7	7
Total (g)	100	100	100

The materials used in this study consisted of tuna fish sourced from fishermen in Puger District, Jember, East Java, Indonesia, and edamame flour obtained from Mitra Tani 27. The chemicals used included distilled water, petroleum benzene, papain enzyme, HCl, selenium, NaOH, H2SO4, H3BO3, methyl red methyl blue indicators, aluminium foil, and filter paper.

Procedure for Making Edamame Flour

The procedure for making edamame flour is based on research by Siregar et al. (2023) with modification (10). The edamame soybeans are soaked in water (3:1) for eight hours. After soaking, the epidermis will easily peel off and separate from the edamame soybean seeds. Next, the edamame soybeans are boiled for 20 minutes. The drying process is done 2 times. First in the sun for 4 hours and then in the oven for 24 hours at a temperature of 60°C. After drying, the edamame soybean seeds are ground and sifted using an 80-mesh Tyler sieve to obtain edamame flour.

Procedure for Making Tuna FPH

The procedure for making FPH is based on research by Witono et al. (2020) (12). Tuna fish is filleted until boneless tuna meat is obtained. After that, the fish flesh is weighed and crushed using a food processor with a ratio of distilled water to fish flesh of 2:1 to the weight of the fish until a suspension is produced. Next, papain enzyme (% v/w) was added in a total of 5% of the weight of the fish meat. Then hydrolysis was done at a temperature of 55°C for 3 hours and continued with enzyme inactivation at a temperature of 85°C for 20 minutes which aimed to stop the hydrolysis process. Next, the tuna fish protein hydrolyzate was centrifuged at 3500 rpm at 10°C for 30 minutes until supernatant and residue were produced. The supernatant obtained was dried using a freeze dryer to obtain dry tuna protein hydrolyzate. The dried tuna protein hydrolyzate is then ground into powder. Based on previous research, the papain enzyme has very high activity in hydrolyzing protein in fish. The papain enzyme has an enzyme activity of 86.32 units/mL, while the calotropin enzyme is only 15.04 units/mL (12). The best concentration for using the papain enzyme in hydrolyzing protein in fish is 5% (13). The papain enzyme can catalyze the hydrolysis process well at temperature and pH conditions within a certain time range because the activity of the papain enzyme is quite specific. The active side of papain consists of amino acids, namely cysteine and histidine. One of the two amino acids, cysteine, is active because it contains a thiol group (-SH) (14).

Organoleptic and Chemical Properties Test

Organoleptic tests include colour, taste, aroma, texture, and overall. The testing method is done by using a liking test using seven scales (very dislike, dislike, somewhat dislike, neutral, somewhat like, like, and very like). The liking test assessment used 25 semi-trained panelists. Chemical properties testing is performed for edamame flour, tuna FPH, and all biscuit formulas. Testing of chemical properties, including proteins (Semimicro-Kjehldal Method, BSN1992), fats (Soxhlet Method, AOAC 2005), and carbohydrates (Carbohydrate by Difference Method).

Data Analysis

The data obtained was analyzed using SPSS version 26. The organoleptic test result data was analyzed using the ANOVA method with a 95% confidence level and if there is a real effect, it will be continued with the post hoc test if there are significant differences between groups.

RESULTS AND DISCUSSIONS

Organoleptic Test

Kruskal Wallis was used to test the results for the organoleptic tests as the data was not normally distributed. If a real difference is found, the Mann-Whitney test is used. The tests cover colour, texture, aroma, taste, and overall results, with the organoleptic test ratios for edamame flour biscuits and FPH of tuna formulas presented in Table 3. Statistical testing shows that only colour parameters have significant differences in at least two groups, while other parameters such as taste, aroma, texture, and overall have no statistically significant differences.

Table 3. Average value of organoleptic test results for edamame flour and tuna fph biscuits

Parameter	F1	F2	F3	*p*
Colour	4.60±0.25[a]	5.68±0.22[b]	5.40±0.21[b]	0.004
Taste	4.64±0.26	4.04±0.32	4.72±0.26	0.218
Aroma	4.28±0.26	3.92±2.58	4.76±0.25	0.053
Texture	4.68±0.31	4.08±0.35	4.44±0.27	0.387
Overall	4.88±0.22	4.36±0.23	5.00±0.20	0.123

Note: The difference annotation letter indicates a significant difference ($p<0.05$), [a,b] Post Hoc Tamhanes

Scale: 1 = very dislike; 2 = dislike; 3 = somewhat dislike; 4 = neutral; 5 = somewhat like; 6 = like; 7 = very like

Preference for Colour

Table 3 shows a significant distinction in colour preference between edamame flour and tuna FPH when utilized in biscuit production. This contrast was observed with a high confidence level of 95%. The preferred colour weighting chosen was F2. The F1 yielded a darker colour with 30% edamame flour and 10% FPH, whereas F2 produced a brighter shade. On the other hand, F3 produced the brightest hue among all formulas. Based on the panelists' evaluation, F2 is the most visually pleasing due to its balanced shade, which is not overly dark or bright. The previous study demonstrates that edamame flour concentration notably impacts snack bar brightness. As the amount of edamame flour increases, the colour becomes darker due to the processing technique, particularly during drying and baking (16). During these procedures, the Maillard reaction can occur, causing the protein's primary amine and the reducing sugar's carboxyl group to react, resulting in a black colour (8).

Preference for Taste

Table 3 displays the statistical analysis of the different formulations of edamame flour and tuna protein hydrolysate, indicating that they do not match the taste preference of edamame flour biscuits and tuna protein hydrolysis at a 95% confidence level. The F3 treatment was used to evaluate the preference level of the chosen flavour. Changes in taste result from several factors, such as chemical compounds, temperatures, concentrations, and interactions with other flavour components (17). During the biscuit-making process, margarine is added (18). Moreover, our study shows that the panels preferred the F3 treatments with higher concentrations of added tuna FPH over other treatments. Tuna fish protein hydrolyzate causes a stronger savory taste due to its protein content. One of the amino acids contained in tuna hydrolyzate is glutamic acid. Glutamic acid is the dominant amino acid in tuna with a content of 12.45% (19). According to previous research, glutamic acid in rebon shrimp hydrolyzate has a role in forming flavor (20).

Preference for Aroma

The statistical test results in **Table 3** showed that the formulations of edamame flour and tuna protein hydrolyzate are not significantly different in terms of preference for the aroma of edamame flour biscuits and tuna protein hydrolyzate at the 95% confidence level. The weighting for the level of preference for the selected aroma is the F3 treatment. In the F3 treatment, there was a higher addition of tuna protein hydrolyzate than in other treatments, resulting in a distinctive aroma. This is in line with previous research that biscuits with the addition of reborn shrimp protein hydrolyzate have a distinctive aroma of reborn shrimp so the higher concentration of reborn shrimp protein hydrolyzate added causes the resulting biscuit aroma to be stronger (21). This is due to the content of amino acids that affected in aroma, which were phenylalanine and tyrosine. The highest essential amino acids contained in tuna fish include lysine (21.04 ± 1.75 mg/g), leucine (17.96 ± 1.11 mg/g), and the lowest is phenylalanine (10.34 ± 1, 63 mg/g) (22).

Preference for Texture

The statistical test results in **Table 3** showed that the formulations of edamame flour and tuna fish protein hydrolyzate are not significantly different in terms of texture preference for edamame flour biscuits and tuna protein hydrolyzate at the 95% confidence level. The selected weighting for the level of texture preference is the F1 treatment. The texture in treatment F1 was preferred by panelists because in this treatment there was a lower addition of protein hydrolyzate than in other treatments. Biscuits with the addition of protein hydrolyzate have a texture that is not crunchy or reduces the crispness of the product (21). The texture of biscuits can be influenced by the raw materials used, baking temperature, and water content (23).

The statistical test results in **Table 3** show that the edamame flour formulation and tuna fish protein hydrolyzate are not significantly different in the texture preferences of edamame flour biscuits and tuna protein hydrolyzate at the 95% confidence level. The weighting chosen for the level of texture preference is the F1 treatment. F1 biscuits have the crunchiest texture among all the treatments. The crunchy texture of F1 biscuits is due to the addition of the least amount of FPH. The addition of FPH to biscuits can affect the crispness of the biscuits. This is in line with previous research which states that the more FPH added to biscuits, the less crispy the product can be (21). In addition, changes in texture can be caused by loss of water or fat content, emulsion breakdown, carbohydrate hydrolysis, and protein coagulation or hydrolysis (24). Other factors that influence the texture are the water content which makes the texture soft, the materials used, the thickness of the mold, and high oven temperatures (22).

Overall

The statistical test results in **Table 3** showed that the formulations of edamame flour and tuna protein hydrolyzate are not significantly different in terms of overall preference for edamame flour biscuits and tuna protein hydrolyzate at the 95% confidence level. Sensory attributes in overall liking include color, aroma, taste, and texture. Based on the results of organoleptic tests on the overall preference of biscuits, it shows that the highest value is F3 biscuits and the lowest value is F2. Overall, the average score of the three biscuit formulations was above 4. This shows that all biscuit formulations were acceptable to the panelists.

Nutritional content of edamame flour and Tuna FPH biscuits

Chemical analysis was done on all formulations, including protein, fat, and carbohydrate levels, while energy level is obtained from the sum of protein, fat, and carbohydrates. Normality test results for protein, fat, carbohydrates, and energy are normally distributed. Based on statistical tests with ANOVA of energy, protein, fat, and carbohydrates in all groups, it shows a p-value<0.001.

Table 4. Comparison of Nutritional Content of Edamame Flour and Tuna FPH Biscuits with Supplementary Feeding Manufactured (100 g)

Parameter	**F1** (mean±SD)	**F2** (mean±SD)	**F3** (mean±SD)	***p***	**Supplementary Feeding Manufactured** (15)
Energy (kcal)	448.75±0.39[a]	443.37±1.51[b]	440.34±0.76[c]	<0.001	464.50
Protein (g)	14.28±0.02[a]	15.72±0.05[b]	16.81±0.07[c]	<0.001	8.45
Fat (g)	21.44±0.05[a]	20.26±0.11[b]	19.66±0.14[c]	<0.001	16.74
Carbohydrate (g)	49.68±0.02[a]	49.54±0.07[a]	49.05±0.06[b]	<0.001	70.00

p < 0.05 significant difference
Note: The difference annotation letter indicates a significant difference (*p*<0.05), [a,b,c] Post Hoc Bonferroni Test

It indicates that there are significant differences in all groups. A comparison of the nutritional content between all formulas of edamame flour and tuna FPH biscuits and supplementary feeding manufactured can be seen in **Table 4**. Based on **Table 4**, all formulas of edamame flour and tuna FPH biscuits have a higher protein and fat content than the supplementary feeding manufactured. This is due to the use of edamame flour and tuna FPH in the biscuits which are known to contain high protein, while the high fat content comes from the added margarine and egg yolk. The carbohydrate content in the edamame flour and tuna FPH biscuits is lower than supplementary feeding manufactured because the wheat flour composition is lower compared to supplementary feeding manufactured. Wheat flour has a higher carbohydrate content than edamame flour (25,26). In the edamame flour and tuna FPH biscuits, wheat flour is substituted with edamame flour so that the carbohydrate content in all formula biscuits is lower when compared to the supplementary feeding manufactured. Meeting the need for adequate energy intake is very important for children. This energy comes from macronutrients such as carbohydrates, fat, and protein. Carbohydrates are the most important energy source for the body. Carbohydrates provide energy for all tissues in the body, especially the brain (27). Protein is an important macronutrient because it contains essential components that cannot be replaced by other nutrients. Apart from its role in supporting children's growth, protein also determines body composition, neurocognitive development, immune system maturity, and organ function. An imbalance between protein needs and protein intake causes stunting in children (28). Fats consumed in food are used as a source of energy and essential fatty acids. Structural fatty acids are an important part of cell membranes, nerve fibers, and cell structure in general. Fat reserves, especially in adipose tissue, are a long-term energy source for the body. Children who receive sufficient energy intake will experience growth and development appropriate to their age, but if there is a lack of energy intake during childhood, this will have an impact on the child's nutritional status (27)

Treatment Selection

The selected formula was determined using the Exponential Comparison Method (ECM). This method aims to determine the priority order of decision alternatives with multiple parameters. The weight is determined based on the featured components of the biscuit.

Table 5. Selected formulas of edamame flour and Tuna FPH biscuits

Parameters	Weight	Component Alternative Scores					
		F1		F2		F3	
		Rank	Score	Rank	Score	Rank	Score
Protein	20%	3	0.6	2	0.4	1	0.2
Fat	15%	1	0.15	2	0.3	3	0.45
Energy	15%	1	0.15	2	0.3	3	0.45
Colour	10%	3	0.3	1	0.1	2	0.2
Taste	10%	2	0.2	3	0.3	1	0.1
Aroma	10%	2	0.2	3	0.3	1	0.1
Texture	10%	1	0.1	3	0.3	2	0.2
Overall	10%	2	0.2	3	0.3	1	0.1
Total	100%		1.9		2.3		1.8
Rank			2		3		1

Rank 1 = highest
Rank 3 = lowest

The parameters considered are protein content (20%), fat and energy (15%, respectively), and all parameters in the organoleptic test (10%, respectively). After that, the weight value is multiplied by the ranking of each parameter in each biscuit formula. The scores for each parameter are then added up to determine the ranking for each formula (29). Based on this description and the calculations carried out for each formula, the formula chosen in this research is the F3 formula. Details can be seen in **Table 5.**

CONCLUSIONS AND RECOMMENDATIONS

The conclusion of this study was the treatment chosen was F3 biscuits with a proportion of 60% wheat flour, 20% edamame flour, and 20% tuna FPH. Based on the chemical properties test, it was found that the protein and fat content of the selected biscuits, F3, was higher than the supplementary feeding manufactured. It is recommended to continue further research to find the right formulation to add local food ingredients as a source of vegetable and animal protein as an alternative for preventing stunting.

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