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Product Formulation of Ready to Use Fish Food (RUFF) from Snakehead Fish Flour (*Channa striata*)

Puspo Edi Giriwono[1,2,✉], Safira P.A. Haryanto[1], Saraswati[1]

[1] Department Food Science and Technology, IPB University, Dramaga, Bogor, INDONESIA.

[2] South East Asia Food and Agriculture Science and Technology (SEAFAST) Center, IPB University, Bogor, INDONESIA

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Corresponding Author:
✉ pegiriwono@apps.ipb.ac.id
(Puspo Edi Giriwono)

ABSTRACT

RUFF (ready-to-use fish food) is a nutrient and calorie-dense food with high protein and energy consumed by children under five years of age who suffer from severe acute malnutrition. RUFF in this study was designed to be the alternative product for RUTF (ready-to-use therapeutic food) using snakehead fish flour as skim milk substitute. This study aims to determine the suitability of snakehead fish flour as an animal protein alternative, obtain the RUFF formula that meets FAO and WHO standards, and determine the consumer acceptance. The research investigated different amount of snakehead fish flour in the RUFF formulation. There were 3 formulas with different snakehead fish flour, i.e. 20% (F1), 22% (F2), and 24% (F3). The results of the analysis showed that the highest protein content was found in formula F3 (14.35 ±0.72)% with moisture content of 2.32 ± 0.06%, ash content of 2.56 ± 0.04%, fat content of 29.39 ± 0.24%, and carbohydrate content of 51.37 ± 0.94%. All three RUFF formulations meet the nutritional standards set by the FAO and WHO. The hedonic rating test results and ranking showed that the most preferred formula by the panelist was formula F1 containing 20% snakehead fish flour with an average rate of 7.46 (like). Formula F1 is the formula of RUFF with the highest rate of consumer acceptance.

1. INTRODUCTION

Malnutrition is a major health problem for children under 5 years of age in low- and middle-income countries. Malnutrition is the cause of 45% of global infant and child deaths each year and causes cognitive impairments for those who survive (Govender *et al*., 2021). Malnutrition is often experienced by children under 5 years of age due to an imbalance of energy and protein, resulting from inadequate nutritional fulfilment. Based on nutritional status monitoring conducted annually by the Ministry of Health, 3.8% of children aged 0-59 months were severely malnourished and 14.0% were mildly malnourished in Indonesia at 2017 (Direktorat Gizi Masyarakat, 2018). Some of the impacts that can occur to children with malnutrition include increased vulnerability to diseases, diminished cognitive abilities, and a gradual decrease in immune function. Therefore, efforts are needed to prevent further complications (World Health Organization, 2019).

Efforts to improve the nutritional status of severely acute malnourished children can be made by improving nutrient intake using ready-to-use therapeutic food (RUTF). RUTF is a recovery food commonly used as home-based rehabilitation for children under five who are malnourished without medical complications and with good appetite (Marzoog *et al*., 2022). RUTF is a high protein and energy food, generally in the form of a thick paste consumed by children with nutritional problems (Kurniasih, 2022). The standard nutritional recommendations for RUTF, according to FAO & WHO (2022), include: energy 520-550 kcal/100 g, protein 10-12% of total energy, fat 45-60% of total energy, and the presence of several micronutrients with predetermined minimum and maximum amounts. The use of

RUTF has been recommended by WHO and UNICEF as a therapeutic food for children experiencing acute malnutrition (Schoonees *et al*., 2013).

The challenge with current commercial RUTF products lies in the use of milk powder as the source of animal protein. Milk powder is a relatively expensive raw material, and its importation is necessary in some regions. In fact, the cost of milk powder alone can account for as much as half of the final price of RUTF. Therefore, there is a need to develop new formulations by incorporating locally abundant foods in Indonesia. As an archipelago rich in fisheries potential, one alternative to using milk powder as a source of animal protein for RUTF is fish. Ready-to-Use Fish Food (RUFF) is a recovery food with fish, nuts, and other ingredients. RUFF is an alternative RUTF product that excludes dairy ingredients, aiming to reduce the cost associated with milk powder used in commercial formulas.

Snakehead fish is a type of freshwater fish that is a popular commodity in Indonesia. It is a source of animal protein that contains all essential amino acids, is highly digestible, and is available in large quantities. Snakehead fish contains high protein levels, reaching 20 g/100 g and has excellent digestibility, reaching more than 90% (Sari *et al*., 2014). Snakehead is processed into snakehead fish flour as a form of semi-finished processed product that can be added to various processed products. Processing snakehead fish into snakehead fish flour is undertaken to enhance the product's durability, ease of incorporation into various food products, and the possibility of enriching it with additional nutrients. The protein content of pure snakehead fish flour can reach 79.62% (Permatasari *et al*., 2021).

The addition of snakehead fish into RUFF is expected to help improve the nutritional intake of foods preferred by children. The wafer roll form with pasta filling has been utilized in local RUTF products and has received positive acceptance from children aged 6-59 months ([UNICEF] United Nations Children's Fund, 2023). This positive response led to the selection of the wafer roll form with pasta filling for the RUFF product. However, the use of fish meal or fish flour presents challenges such as a decrease in sensory acceptability from the distinct fish flavors. Therefore, this study aims to develop and formulate a more sensory acceptable RUFF products with snakehead fish flour as an alternative to land based animal protein in RUTF, can meet the nutritional RUTF composition standards of FAO & WHO (2022), and produce products that are accepted by consumers. Foreseeable benefits from the formulation and development of this RUFF product include lower dependence in dairy protein import, utilizing locally sourced more sustainable protein from fish to address nutritional problems in children, and possible development in aquaculture based local economy.

2. MATERIALS AND METHODS

2.1. Material

The materials used in this study were commercially available snakehead fish flour (PT. XYZ), peanut butter (Beorganik), vegetable oil (Filma), chocolate powder (Van Houten), isolate soy protein (ISP) (Linyi Shansong), sugar (Rose Brand), texturizer (PT. XYZ), vitamin-mineral premix (DSM, Malaysia), and wafer stick roll (Kogen). Chemical reagents used for proximate analysis include hexane (Merck), HgO (Merck), K_2SO_4 (Merck), H_2SO_4 (Merck), distilled water (Bratacco), NaOH-$Na_2S_2O_3$ (Merck), H_3BO_3 (Merck), methyl red and methylene blue indicators (Merck), HCl (Merck).

2.2. Methods

2.2.1. Snakehead fish flour production

Snakehead fish flour was sieved with an 80-mesh sieve to prevent clumping. Dry ingredients (snakehead fish flour, sugar, and cocoa powder) were homogenized with a blender for 30 seconds until all dry ingredients were well mixed. Peanut butter and vegetable oil were added and homogenized with a blender again for 30 seconds until all ingredients were oil soluble and homogeneous. Texturizer, ISP, and vitamin-mineral premix were added last to the mixture while being homogenized at low speed to yield stable and homogenous texture. Temperature of homogenization was measured and reached a maximum of 45°C. The thickened RUFF paste is put into a wafer stick roll using a filling syringe. The ratio of RUFF paste to wafer stick roll was 2 : 1 or 67% and 33% for a total of 100 g. Fully Randomized Design was used, and each treatment (different quantity of snakehead fish flour) was replicated 3 times.

2.2.2. RUFF formulation

RUFF formulations were made by considering nutritional calculations that meet the nutritional composition standards per 100 g of RUFF according to FAO & WHO (2022) with alternative animal protein from snakehead fish flour. There were three formulas, namely F1 with 20 g snakehead fish flour, F2 with 22 g snakehead fish flour, and F3 with 24 g snakehead fish flour. The amount of sugar was adjusted so that the RUFF paste formula could reach 100 g. RUFF paste formula per 100 g of each formula is presented in Table 1. Production of each formula was replicated 3 times.

Table 1. RUFF paste formulation per 100 g

Formulation	Unit	F1	F2	F3
Snakehead fish flour	g	20	22	24
Sugar	g	10	8	6
Chocolate powder	g	5	5	5
Peanut butter	g	30	30	30
Vegetable oil	ml	26	26	26
Texturizer	g	2	2	2
Isolate soy protein (ISP)	g	5	5	5
Vitamin-mineral premix	g	2	2	2
Total	g	100	100	100

2.2.3. Amino Acid Sufficiency

Amino acid sufficiency pattern analysis was conducted on RUFF protein sources, namely snakehead fish flour, peanut butter, and isolate soy protein (ISP) using a literature study. Analysis of amino acid sufficiency patterns in RUFF protein sources was carried out to measure whether RUFF essential amino acids have met the amino acid composition standards according to the WHO (2021).

2.2.4. Proximate and Sensory Analysis

RUFF was analyzed for its proximate values (AOAC, 2005) and sensory evaluation. Sensory evaluation was carried out using hedonic and ranking tests. Hedonic test was used to measure the level of favorability of the product. The number of panelists involved was 50 people who were untrained panelists with the conditions of age above 17 years, not having impaired vision, taste, smell, and not having allergies to fish and nuts. A hedonic rating test was conducted to measure the liking level of RUFF. The scale used was a numerical scale of 1-9, namely: (1) very strongly dislike, (2) very dislike, (3) dislike, (4) somewhat dislike, (5) average, (6) somewhat like, (7) like, (8) very like, and (9) very strongly like. The sensory attributes tested were filling color, aroma, taste, texture, aftertaste, and overall attribute at the 5% significance level. The ranking test was conducted to rank the panelists' level of liking for RUFF and determine the preferred RUFF formula. Panelists were asked to rank the most to the least preferred RUFF formula. The lowest number indicates the highest level of liking while the highest number indicates the lowest level of liking.

2.3. Data Analysis

Statistical analyses were conducted using one-way analysis of variance (ANOVA), followed by Duncan's post hoc or Least Significant Difference (LSD) test to identify differences at a significance level of 5%, where appropriate. For the hedonic ranking test, the LSD method was applied, as it is more suitable for sensory evaluation than Duncan's multiple range test. LSD provides sensitive pairwise comparisons, allowing subtle differences in panelists preference to be detected. All analyses were performed using SPSS software, version 25.0 (IBM Corp., Armonk, NY, USA).

3. RESULTS AND DISCUSSION

3.1. Proximate Composition of Commercial Snakehead Fish Flour

Commercial snakehead fish flour is produced and available commercially in Indonesia, and claimed to contain protein

levels of 20 – 30%. The proximate results of the nutritional content of snakehead fish flour can be seen in Table 2. The proximate results showed that the protein content of snakehead fish flour was only 23.56% and had a high carbohydrate content reaching 67.75%. This is not in accordance with the statement of (Sari *et al*., 2014), who reported that the protein content of snakehead fish flour could reach 76.9% and the carbohydrate content of snakehead fish flour was 3.53%. The elevated protein content observed in the study conducted by (Sari *et al*., 2014) is attributed to the use of fresh snakehead fish edible parts, particularly the muscle, which is recognized for having the highest protein content among snakehead fish components (Prastari *et al*., 2017). In this study, the utilized commercial snakehead fish flour was presumed to possess decreased fish muscle content, potentially employing high-carbohydrate flours as fillers. Sumarto *et al*. (2021) reported that the production of composite flour from fish and starchy flour can enhance product diversification. The incorporation of 10% fish flour with about 72% protein content (Ilisha elongate) to sago flour will result in a composite flour with carbohydrate and protein content of 83.23% and 6.39%, respectively.

Table 2. Proximate values of commercial snakehead fish flour

Component	Amount (%)
Water	1.86±0.03
Ash	3.50±0.04
Protein	23.56±0.16
Fat	3.34±0.29
Carbohydrate	67.75±1.13

3.2. Proximate Composition of RUFF

Based on the analysis results, the three RUFF formulas have a moisture content with a range of 2.32% – 2.36% and meet the value requirements set by FAO & WHO (2022), which is a maximum of 2.5%, as shown in Table 3. The raw materials used are powdered with low moisture content, in addition to vegetable oil added that also has no water content. The highest moisture content is wafer stick roll at 3.90%, but this value is still below the basic characteristic value of wafers required by Badan Pengawas Obat dan Makanan (2022), which limits moisture content to no more than 5%. The ash content of the three formulations was in the range of 2.56% to 2.62%. This value was assumed to be the total mineral content contained in RUFF, but no specific mineral analysis was conducted. The source of minerals in the RUFF formula comes from a vitamin-mineral premix that is considered to meet the FAO & WHO (2022).

The protein content of the three formulas was in the range of 13.03% – 14.35% and meets the value requirements set by FAO & WHO (2022). The protein sources in the RUFF formula came from peanut butter, snakehead fish flour, and ISP. Peanut butter is well-known to be rich in protein, containing at 24.9 g/100 g (Kusuma *et al*., 2016). The small differences in protein content were mainly attributed to the differences in the amount of snakehead fish flour that was used in each of the formulas, indicating there was significant differences detected by ANOVA.

Table 3. Proximate values of RUFF per 100 g

Component	F1	F2	F3
Moisture (%)	2.36 ± 0.02	2.35 ± 0.11	2.32 ± 0.06
Ash (%)	2.59 ± 0.17	2.62 ± 0.11	2.56 ± 0.04
Protein (%)	13.03 ± 0.06	13.42 ± 0.07	14.35 ± 0.72
Fat (%)	29.17 ± 0.37	29.28 ± 0.14	29.39 ± 0.24
Carbohydrate (%)	52.86 ± 0.39	52.33 ± 0.76	51.37 ± 0.94
Energy (kCal)	526.06	526.52	527.45

Notes: Values are mean + SD, n = 3. No significant differences were detected between formulas.

The inclusion of significant amounts of peanut butter designates it as one of the primary protein sources in the RUFF formula. To provide high quality protein, approximately 22-24% concentration of snakehead fish flour was incorporated into the RUFF formula to mitigate the emergence of a strong fishy aroma and to lessen the intensity of the yellow color. The study of (Afianti & Indrawati, 2015) showed that incorporating up to 30% snakehead fish flour

into the cracker formulation might impart a distinct fishy flavor. In addition, ISP also contributed to increasing the protein content in the RUFF formula. ISP is the purest form of protein from soybeans with a minimum protein content of 90% obtained by extracting soluble proteins and removing materials other than proteins such as fats and carbohydrates (Astawan & Prayudani, 2020). ISP has a neutral flavor and is widely used to improve the quality of food products, including the manufacture of infant formula.

The fat content of the three formulas was in the range of 29.17% – 29.39% and meets the value requirements set by FAO & WHO (2022). Fat sources in the RUFF formula came from vegetable oil and peanut butter. Vegetable oil was chosen because it is cheap and available in large quantities, making it suitable for use in RUFF products that contain high fat. Peanut contains high fat, namely unsaturated fatty acids in the form of oleic acid (C18:1) and linoleic acid (C16:2 omega 6), as well as saturated fatty acids consisting mainly of palmitic acid (C16:0) and myristic acid (C14:0) (Kusuma *et al*., 2016). The carbohydrate content of the three formulas fell within the range of 51.37% to 52.86%. In RUFF, carbohydrates play a crucial role in balancing the fat and protein content, contributing to its overall high energy content as highlighted by (Mentari *et al*., 2022). The primary source of carbohydrates was discovered to be commercial snakehead fish flour, containing 67.83% carbohydrates. The high content of carbohydrate observed in this commercial snakehead fish flour indicates that filler of starch or polysaccharide sources were added to this flour, indicating that these commercial fish flour are not purely made from fish meat but added with lower price starch. Additionally, the wafer also contributes to the total carbohydrate content, given its composition that includes a relatively high amount of wheat flour.

3.3. Pattern Analysis and Nutritional Adequacy of RUFF Amino Acids

Amino acid pattern of RUFF's raw material can be seen in Table 4. The amino acid pattern data were obtained from the literature and subsequently utilized to project the amino acid composition in the formulated RUFF. The calculation results can be seen in Table 5, showing that the amino acid composition of the three RUFF formulas still does not meet the prescribed amino acid pattern of RUTF. The homogenization step of all ingredients in producing the RUFF paste resulted in a maximum temperature reaching 45°C, which may account to loss of some the heat sensitive amino acids. This may be addressed by recommendation from FAO (2018), that commercial RUFF made from 25% skimmed milk (more than 50% of the protein comes from milk) can meet the established amino acid standards. In 100 g of RUTF with a skim milk and peanut butter as protein sources, 849 mg lysine, 553 mg SAA, 517 mg threonine and 178 mg tryptophan are contained (FAO, 2018). This shows that the pattern and number of amino acids in the RUFF formula are lower than the commercial RUTF, so the use of snakehead fish flour as animal protein still cannot replace skim milk in the pattern and adequacy of amino acids for the recovery of toddlers with severe acute malnutrition. This can be related the low amount of protein content of commercial snakehead fish flour used in this study.

Table 4. Amino acid pattern of raw materials serving as the protein sources

Amino acid (mg/g)	Snakehead Fish (Prastari *et al*., 2017)	Peanut Butter (Dharsenda *et al*., 2020)	ISP (Mohsen *et al*., 2009)	Casein (Liu *et al*., 2019)
Histidine	7.8	6.2	27	31
Isoleucine	20.2	8.7	43	59
Leucine	30.8	15.9	78	102
Lysine	27.3	8.8	65	85
SAA*	0.5	6.2	28	33
AAA**	16.4	22.8	54	114
Threonine	0.5	8.4	36	46
Tryptophan	0	2.4	10	14
Valine	19.1	10.3	45	76

*SAA = sulfur amino acids (methionine + cysteine)
**AAA = aromatic amino acids (phenylalanine + tyrosine)

Table 5. Estimation of the amino acid pattern and amount of amino acids in the formulated RUFF based on literatures as mentioned in Table 4.

Amino Acid	AA requirements for optimum child growth (mg/g) (FAO, 2018)	F1 Total (mg/g protein)	F2 Total (mg/g protein)	F3 Total (mg/g protein)
Histidine	24	12.27	12.15	12.03
Isoleucine	34	21.20	21.17	21.15
Leucine	70	36.86	36.70	36.54
Lysine	65	29.18	29.13	29.08
SAA	31	10.48	10.21	9.94
AAA	63	29.43	29.08	28.73
Threonine	36	13.62	13.27	12.92
Tryptophan	10	3.77	3.67	3.57
Valine	46	22.15	22.07	21.99

3.4. Hedonic Rating Results of RUFF

Based on the hedonic test results on the filling (RUFF paste) color attribute, the average value of liking ranged from 7.50 – 7.60, indicating that panelists tended to like the three filling colors of the RUFF formula, shown in Table 6. The outcomes of the ANOVA test revealed that there was no significant difference in the liking levels of the sensory attributes related to filling color among the various formulas ($p > 0.05$). Color can be influenced by product raw material components and the product manufacturing process (Khalisa *et al*., 2021). The color of the RUFF formula filling tends to be brown due to the addition of chocolate powder in each formula.

Table 6. Average favorability score based on hedonic rating test

Attributes	F1	F2	F3
Filling/Paste Color	7.54±1.20 ^{a}	7.60±1.05 ^{a}	7.50±1.22 ^{a}
Aroma	7.20±1.54 ^{a}	7.14±1.14 ^{a}	7.48±1.13 ^{a}
Taste	7.46±1.16 ^{a}	7.18±1.19 ^{ab}	6.90±1.50 ^{b}
Texture	7.68±1.32 ^{a}	7.44±1.28 ^{a}	7.62±1.24 ^{a}
Aftertaste	7.34±1.36 ^{a}	7.00±1.39 ^{a}	6.80±1.60 ^{a}
Overall	7.54±0.95 ^{a}	7.44±0.88 ^{a}	7.14±1.29 ^{a}

Notes: Values are Mean ± SD; n = 3; letters in the same row indicate insignificant different ($p \geq 0.05$) according to Duncan's posthoc test

Based on the hedonic test results for the sensory attributes of aroma, the average liking values ranged from 7.14 to 7.48, suggesting that panelists generally favored the three aromas of the RUFF formula. The ANOVA test for aroma attribute yielded a p-value>0.05, indicating no significant difference in the liking levels of aroma sensory attributes among the formulas. It's worth noting that the aroma in the RUFF formula is primarily influenced by the dominant peanut aroma, given the use of peanut butter as a key raw material. The complexity of aroma perception arises from the combination of various odorous compounds, creating a distinct impression that differs from the individual aromas of its components (Khalisa *et al*., 2021).

Based on the hedonic test results on the taste attribute, the average value of liking ranged from 6.90 – 7.46, indicating that panelists tended to like the three flavors of RUFF formula. The ANOVA test results showed that there was no significant difference in the level of liking of the taste sensory attributes between the formulas. However, further tests (Duncan) show that formula F1 is significantly different from formula F3, while formula F2 is not significantly different from F1 and F3. RUFF formula has a dominant flavor of sweet chocolate and peanut, with a slight salty and bitter taste. The peanut flavor comes from peanut butter and the sweet flavor comes from the addition of sugar. Incorporating snakehead fish flour influences both the salty and savory flavor components in the RUFF formula. As the amount of snakehead fish flour increases, the taste of the RUFF formula becomes progressively saltier and more savory.

Based on the hedonic test results on the texture attribute, the average value of liking ranged from 7.44 – 7.68, indicating that panelists tended to like the three textures of the RUFF formula. The ANOVA test for texture yielded a p-value > 0.05, indicating no significant difference in the liking levels of texture attributes among the formulas. The key textural elements in food, notably, softness and crunchiness, are crucial. Attributes such as hardness, cohesiveness, and water content are frequently taken into account (Lamusu, 2018). In the RUFF formula, the wafer imparts a crunchy texture, while the filling exhibits a soft, paste-like consistency.

Based on the results of the hedonic test on the sensory attributes of aftertaste, the average value of liking ranged from 6.80 – 7.34, indicating that panelists tended to like the aftertaste of the three RUFF formulas. Different RUFF formulas did not affect the results of hedonic test to the aftertaste attribute. Consumers generally do not prefer the taste and aroma of fishiness (Sarastani *et al*., 2023). The RUFF formula leaves an aftertaste characterized by both saltiness and bitterness. The presence of cocoa powder contributes to a bitter aftertaste, potentially masking the salty or fishy flavor of the snakehead fish flour.

The overall acceptance of the RUFF formula was assessed based on the results of observations on the combination of the five sensory attributes, namely filling color, aroma, taste, texture, and aftertaste. The ANOVA test results for overall acceptance have a range of average favorability scores ranging from 7.14 – 7.54, indicating that panelists tend to like the overall RUFF formula. Generally, the ANOVA test indicates that there is no significant difference in overall acceptance of the RUFF formulas.

3.5. Ranking Test Results

The ranking test was conducted to determine the RUFF formula that was most preferred by the panelists. The ranking test data was analyzed using the Friedman test. The smaller the average value, the higher the ranking. The results of the RUFF ranking test can be seen in Table 7, showing that the average value from the lowest to the highest was formula F1 at 1.54 points, then formula F2 at 2.10 points, and formula F3 at 2.36 points. Formula F1 was formulated with a minimal amount of snakehead fish flour implying a reduced salty aftertaste. Additionally, the higher quantity of sugar influences the sweetness level of RUFF products. The conclusion drawn from the lowest average value is that Formula F1 is the RUFF formula most preferred by the panelists.

Table 7. Ranking test results

Formula	Average value	Ranking
F1	1.54 ± 0.73^{a}	1
F2	2.10 ± 0.79^{b}	2
F3	2.36 ± 0.72^{b}	3

Notes: letters in the same column indicate not significant difference according to the LSDrank test

4. CONCLUSIONS

Commercial snakehead fish flour contains 23.56% protein which can be used as an alternative to milk protein in RUFF formulations. There are three RUFF formulas with the amount of snakehead fish flour starting from 20 g, 22 g, and 24 g to achieve the desired nutrition. The results of proximate analysis showed that F1 contained protein content of 13.03%, F2 contained protein content of 13.42%, and F3 contained protein content of 14.35%. The three RUFF formulas met the nutritional composition standards of RUTF required by WHO. However, the amount of some amino acids did not meet the standards set by FAO. The results of hedonic rating and ranking analysis showed that formula F1 was the most preferred formula with the composition of 20% snakehead fish flour, 10% sugar, 5% cocoa powder, 30% peanut butter, 26% vegetable oil, 2% texturizer, 5% ISP, and 2% vitamin-mineral premix. The formula with the composition of 20% snakehead fish was found to be the preferred formula by panelists. Further study would be valuable in combining snakehead fish protein with other protein-rich ingredients, in order to improve the amino requirements of the product.

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