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Research Article

The Potential of Biscuit Fish Protein Hydrolysate Biscuits as an Alternative Protein Source Snack for Toddlers

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ABSTRACT

This study aims to evaluate the influence of substituting Fish Protein Hydrolysate (FPH) on the total proximate and amino acid compositions of biscuits. This experimental method used a single-factor Completely Randomized Control (CRD). Three variations in the ratio of FPH to wheat flour were tested: F1 (85% wheat flour:15% FPH), F2 (83% wheat flour:17% FPH), and F3 (80% wheat flour:20% FPH), along with one control formulation containing 100% wheat flour. Other ingredients used in this study were tapioca flour, margarine, isolated soy protein, egg yolk, skim milk, powdered sugar, baking powder, salt, vanilla, and marie essence as the source of the distinctive aroma of marie biscuit. A comparative evaluation of the nutritional content and amino acid composition in the biscuits was performed using One-Way Analysis of Variance (ANOVA) in SPSS27. The nutritional value analysis demonstrated substantial differences across the groups ($p<0.05$); the higher the amount of FPH, the higher the nutritional value, amino acid, Essential Amino Acid Score (EAAS), protein digestibility, and Protein Digestibility-Corrected Amino Acid Score (PDCAAS) content of the other formulations. In F3, the protein (14.86%), total fat (15.6%), aspartic acid (8,932.48 mg/kg), leucine (11,377.885 mg/kg), lysine (3,955.64 mg/kg), protein digestibility (78.30%), and PDCAAS (5.4%) contents were the highest compared to those in the other formulations (F0, F1, and F2). These results imply that F3 is the most nutritionally enhancing snack, making it a suitable alternative for toddlers' diets.

INTRODUCTION

Childhood, especially from birth to 3 years, is a crucial period for determining the direction of children's growth and development. However, during this period, children have many vulnerabilities, particularly in terms of nutritional intake (Steenbergen *et al.* 2021). Therefore, children must obtain optimal delivery intake between the ages of 1 and 3 years, especially in terms of protein. Protein is a macronutrient compound that plays a key part in deciding the course of children's growth and development (Novianti & Parrangan 2022).

Proteins are vital for the body's growth process, including the formation of new tissues during growth and development, maintenance, repair, and replacement of damaged tissues, and provision of amino acids. Protein can be found in plants and animals; however, animal protein is better than vegetable protein because the body can absorb animal protein almost 90−99% per 100 g of food (Sari & Fadhilah 2021).

Fish are rich in nutrients, particularly proteins. The protein content in fish reaches 15−24% with fat content of 0.1−22%, Docosahexaenoic Acid (DHA) 6.1−10.3%, and Eicosapentaenoic Acid (EPA) 3.7−4.5% (Ali *et* *al.* 2022). In another study, fish has eight essential amino acids 18−20% protein (Maulu *et al.* 2021). Omega-3 fatty acids and proteins are abundant in a variety of fish, and increasing intake of local fish will benefit both children's nutritional status and the local economy (Diana *et al.* 2020).

Unfortunately, so far, the only fish that have been utilized are those with a high selling value, are well-shaped and tac, and are large (Kasthuri *et al.* 2021). Small fish that are accidentally caught become waste fish (bycatches), known as trash fish or fish waste (Diamahesa *et al.* 2023).

However, discarded fish also have good nutritional content. Based on several studies that have been conducted before, the protein content of the waste fish can reach 14 g/100 g of fish (Mardiyah *et al.* 2024). Another study found that the protein content in low-quality fish reached 50−70% (Diamahesa *et al.* 2023). Therefore, it is possible to process them into foods with high potential.

Fish waste can be used to prepare fish protein hydrolysates. Fish Protein Hydrolysates (FPH) are results of the enzymatic degradation of fish proteins, specifically targeting peptide bonds to produce peptides with shorter chains or simpler amino acids. This makes them more easily digestible because of their high bioavailability, which can help increase the protein intake in children (Gao *et al.* 2021).

Based on research from Yuniarti *et al.* (2025), the protein content in fish protein hydrolysates ranges from 60−90%. In addition, according to Mardiyah *et al.* (2024), the protein content of FPH was 82.06%. This indicates that FPH has excellent potential for addressing malnutrition.

Several factors may be related to the lack levels of protein consumption in children including an inadequate diet. As stated by the Food and Agriculture Organization of the United Nations (FAO), protein consumption in Indonesia remains relatively low at only 20 g/day (Sholikhah & Dewi 2022). This is exacerbated by the large number of snacks for children that are abundant in fats, carbohydrates, and sugar but lacking in protein (Lazou 2024).

According to the Indonesian National Standard (SNI) number 2973:2022 concerning biscuits, biscuits are made using wheat flour and oil/fat with or without the addition of additional ingredients. In general, biscuits are made from wheat flour and oil or fat, which have a crispy texture due to the baking process (SNI 2022). Meanwhile, the protein content in wheat flour is only 10.9 g per 100 g (USDA 2020); therefore, it is necessary to innovate snacks that children like, with better nutritional content that still taste good

One effort that can be made is to add FPH to biscuits, which is expected to be an innovation in the food sector that increases protein intake in children. Therefore, this study aims to investigate the nutritional composition of biscuits substituting HPI for a substitution for wheat flour, which could be used as an alternative source of protein for toddlers.

METHODS

Design, location and time

This study adopted a Completely Randomized Experimental Design (CRD) to explore the implications of adding FPH on the nutritional value, amino acid compositions, and protein digestibility of biscuits. This study involved four formulations: F0 (100% wheat flour:0% FPH), F1 (85% wheat flour:15% FPH), F2 (83% wheat flour:17% FPH), and F3 (80% wheat flour:20% FPH). The nutritional value and amino acid content of the product were analyzed at SIG Laboratory (PT. Saraswati Indo Genetech, Semarang, Indonesia). Furthermore, protein digestibility testing is conducted on Lab. Chem-Mix Pratama, Yogyakarta, Indonesia. Ethical approval was granted by the Faculty Ethics Review Committee (ref. no: 108/EC/KEPK/FK-UNDIP/V/2025).

Material and tools

The materials utilized for the research include FPH (Berikan Protein) from rucah fish packed in metalized plastic vacuum and stored in refrigerator, wheat flour (Segitiga Biru®) 20 g, tapioca flour (Pak Tani Gunung) 13.3 g, margarine (BlueBand®) 17 g, Isolate Soy Protein (ISP) (Para Agribusiness) 1.3 g, egg yolk 2 g, skim milk (IndoPrima) 3.3 g, sugar (Gulaku) that is pulverized 6.7 g, baking powder (Koepoe-Koepoe®) 0.3 g, salt (Kapal) 0.1 g, vanilla (Mobil) 0.1 g, and marie's essence (Tova) 0.2 g as the source of the distinctive aroma of marie biscuit. The tools used in this study included containers, “Ohaus” analytical scales, trays, an oven from “Vienta,” a rolling pin, and a cookie cutter.

The chemicals used in this study were hexane solvent, K2SO4, CuSO4, concentrated

H_2SO_4, 45% NaOH, 4% H_3BO_3, 0.1 N HCl, 1 N NaOH, ethanol, acetone, distilled water, 15 mL 0.1 N HCl with pepsin enzyme, 0.5 N NaOH, pancreatin enzyme, 0.2 M phosphate buffer solution, 0.005 M sodium azide, casein, biscuits with distilled water reagent pH 8.0, 1 N NaOH, 0.1 M TCA, NO_2CO_3, Folin reagent, enzyme mixture (1.6 mg trypsin with + 3.1 mg chymotrypsin + 4 mg pancreatin per ml distilled water or phosphate buffer pH 8.0), TCA 0.1 M, Na_2CO_3 0.4 M, and 50% Folin reagent (30 mL folin + 60 mL distilled water).

Procedures

The four biscuit formulations were prepared by weighing all the ingredients and mixing them by adding the wet ingredients first such as egg yolk, margarine, vanilla, and marie's essence, followed by the dry components, such as flour, FPH, ISP, skim milk, salt, baking powder, and sugar. The ingredients were combined equally and shaped into a mold. The final stage involves baking the biscuits in a heated oven at 150 °C for around 10 min. Subsequently, each biscuit formulation underwent proximate and amino acid testing.

The protein content was analyzed with the Kjeldahl technique. A 1 g of the sample was weighed and inserted into a digestive tube along with a Kjeltabs Cu 3.5 catalyst tablet and 15 cc of sulfuric acid. The tube underwent heating in the KJEDATHERM system at 380°C for a duration of 2 hours and 50 minutes for digestive purposes. Subsequent to digestion, a tube and a flask containing 25 mL of 1% boric acid and 4 drops of methyl red indicator were positioned within a distillation apparatus. The machine autonomously administered 70 mL of sodium hydroxide and 50 ml of distilled water. The resultant distillate was subsequently titrated with hydrochloric acid until the solution exhibited a pink hue. The volume of acid utilized was documented. Let A represent the volume of HCl utilized for the sample and B for the blank. The nitrogen and protein concentrations were subsequently determined using a specified formula.

$$Nitrogen\ (\%) = \frac{0.1 \times (A - B) \text{ X } 100}{Weight\ sample\ (g) \text{ X } 1000} \text{ X } 100$$

$$Protein\ (\%) = Nitrogen\ (\%) \text{ X } 6.25$$

The fat percentage was evaluated utilizing Soxhlet method. A 5 g sample were weighed and placed in a beaker. Subsequently, 10 mL of hydrochloric acid was injected, and the mixture was subjected to boiling for 30 minutes. The sample underwent filtration, then the beaker was rinsed with distilled water to deposit any leftover residue onto the filter paper. The filter paper containing the residue was desiccated overnight. The extraction beaker was cleaned, dried, and weighed prior to the addition of a cotton wool piece to the thimble, which was thereafter positioned in the beaker. Approximately 150 mL of petroleum ether was introduced, and the beaker was positioned into the SOXTHERM apparatus. Subsequent to extraction, the beaker was subjected to drying in an oven at 105°C overnight, followed by weighing to ascertain the fat content. The fat percentage was determined as follows:

$$Fat\ (\%) = \frac{(W3 - W2)}{W1} \text{ X } 100$$

W1=weight of the sample, W2=weight of the empty extraction beaker, and W3=weight of the extraction beaker with fat.

The moisture level was evaluated using the gravimetric technique. The crucible was initially dried in the oven for 15 to 30 minutes, subsequently chilled in a desiccator, and then weighed. Approximately 5 g of samples were added, and the starting measurement was documented. The crucible itself was subsequently dried in the oven at 105°C overnight then reweighed.

$$Moisture\ (\%) = \frac{(M3 - M2)}{(M1 - M)} \text{ X } 100$$

M indicate the weight of the empty crucible, M1 denote the weight of the crucible plus the sample prior to drying, and M2 signify the weight of the crucible plus the sample subsequent to drying.

The amount of ash was analyzed using the dry ashing method. An empty crucible was first dried, cooled, and weighed. Then 2 g of sample was added to crucible and placed in a muffle furnace at 550°C overnight. Subsequent to ashing, the crucible was cooled and reweighed.

$$Ash\ (\%) = \frac{(W1 - W0)}{(W1 - W0)} \text{ X } 100$$

W0=weight of empty crucible, W1=weight of crucible with ash and W2=weight of crucible with sample.

The percentage of carbohydrates was determined utilizing the difference method Additionally, the amino acid content will be tested using High-Performance Liquid Chromatography (HPLC), as outlined in the Official Methods of Analysis of AOAC International 2023. Amino acid analysis involves the degradation of proteins with 6N HCl to release amino acids. The hydrolysate is solubilized with sodium citrate buffer, and each amino acid is purified using HPLC. Protein extraction is performed using the Kjeldahl method before hydrolysis.

The essential amino acid score was calculated using the FAO/WHO suggested pattern of amino acid requirement for young children (6 months to 3 years) (FAO/WHO 2013). The calculations are as follows:

$$EAAS = \frac{content\ EAA\ in\ sample\ (mg/g)}{content\ EAA\ in\ reference\ (mg/g)}$$

Protein digestibility was tested using an in vitro method with analysis of the reduction in protein pH that happened following the hydrolysis reaction. A total of 0.5 g gs of biscuit powder and standard casein were taken and added to 30 mL of distilled water with a pH of 8.0, then mixed until homogenous. A total of 20 mL of the homogenous mixture was taken to be utilized as a blank and treatment. The treatment solution was mixed with 1 mL of enzyme solution. Then, both the blank and treatment solutions were incubated for 10 minutes at 37°C. A 2 mL of the incubated solution was then taken and placed in a test tube, while the rest was assessed for pH following incubation. Up to 4 mL of 0.1 M TCA was added to the 2 mL of solution in the test tube, which was then vortexed and centrifuged at 3,500 rpm for 10 minutes. Later, 1.5 mL sample of the supernatant obtained from centrifugation was taken and 5 ml of Na2CO3 and 1 mL of folin were added. The solution was subsequently incubated for 20 minutes at 37°C; finally, the amount of absorbance was calculated using a spectrophotometer at a wavelength of 578 nm (Handayani & Sarwono 2021). Meanwhile, for the PDCAAS is calculated using the following formula:

$$PDCAAS = \frac{limiting\ EAA}{content\ EAA\ in\ reference} X\ protein\ digestibility$$

Data analysis

The research data were analyzed using One-Way ANOVA in SPSS 27 (IBM, New York, USA) to evaluate the effect of adding FPH on the nutritional value. Tukey's HSD post-hoc test was used to analyze differences among groups of all nutritional value, protein digestbility, and amino acid parameters (except glutamic acid) because the coefficient of variation was <5%. Duncan's post-hoc test was used to analyze the glutamate acid because of a coefficient of variation >10%. The results of the nutritional value analyses showed significant differences among the groups ($p<0.05$), and each sample was replicated twice (n=2).

RESULTS AND DISCUSSION

Based on the Regulation of the Head of the Food and Drug Monitoring Agency No. 9 year 2016 on Nutrition Label Reference, protein source food claims must meet at least 20% of the daily protein needs from the nutrition label. The daily protein requirement for children between the ages of 1 and 3 years, according to the nutritional label reference, is 26 g/day. This means that 100 g of product must contain at least 5.2 g of protein to meet 20% of the daily protein requirement (BPOM 2016).

Table 1 shows that the protein content in all formulations (F0, F1, F2, and F3) met the minimum requirements for protein source claims because it ranges from 9.24−14.87 g/100 g (the protein percentage is calculated from 100 g of material, so that every 1% is equal to 1 g). Therefore, all biscuit formulations can be considered as are protein source biscuits because they meet at least 20% ALG/100 g in solid form. The highest amount of protein was generated in F3, which was 14.87 g (14.87±0.06).

ANOVA test results for protein content revealed a significant difference between F3 and the other three formulations ($p<0.05$). This is in line with the research conducted by Resti *et al.* (2024), who found that the higher the addition of FPH to food products will result in higher protein content in product.

Proteins are essential components required by toddlers to synthesize new tissues during growth, and reduce enzymes and hormones that regulate various physiological processes in the body (Rofidah *et al.* 2024). Therefore, it is important for toddlers to consume protein-rich food. The fat content of the biscuit samples (Table 1) shows that F3 had the highest fat content (15.6±0.09). This indicates an increase in the fat content of F1, F2, and F3. The difference in fat content also increased significantly ($p<0.05$) for each formulation. The difference in fat content in food can be affected by multiple factors,

Table 1. Analysis of nutritional value in 100 g biscuit substitute FPH

Parameter	Formulation (%) (mean±SD)				p
	F0	F1	F2	F3	
Protein	9.24±0.17[a]	9.2±0.03[a]	9.35±0.17[a]	14.87±0.06[b]	0.000
Fat	13.45±0.06[a]	11.8±0.04[b]	12.6±0.14[c]	15.6±0.09[d]	0.000
Carbohydrate	70.22±0.33[a]	69.52±0.16[a]	66.18±0.47[b]	61.02±0.5[c]	0.001
Ash	2.12±0.04[a]	2.14±0.02[a]	2.08±0.03[a]	1.96±0.01[b]	0.011
Water	4.97±0.05[a]	7.36±0.08[b]	9.8±0.13[c]	6.56±0.53[b]	0.000

Tested using One-Way ANOVA; mean±SD in the same row with different [a,b,c,d] indicates significant difference on further test; Following post hoc test using Tukey HSD; F0 (0% FPH); F1 (15% FPH); F2 (17% FPH); and F3 (20% FPH); FPH: Fish Protein Hydrolysate; SD: Standard Deviation

including the ingredients utilized and the cooking process employed (Adawyah *et al.* 2020).

Table 1 shows a significant downward trend in carbohydrate content as the amount of FPH added increases (p>0.05). This indicates that the higher the amount of FPH added, the greater the reduction in carbohydrate content, because the addition of FPH replaces some of the carbohydrates contained in wheat flour (Hashem *et al.* 2023). Carbohydrates play an important role in giving biscuits their firm, crisp texture and preventing them from crumbling easily.

The results of ash testing for each formulation demonstrated that the incorporation of FPH had no impact (p>0.05) on the ash content of the biscuits. Table 1 shows that the ash content produced was in the range of 1.92−2.14%, indicating that none of the formulations reached the maximum ash content specified in the Indonesian National Standard, which is 1.5% (SNI 2022). The ash content is the amount of residual combustion of inorganic substances in the test product (Yuniarti *et al.* 2025).

Moisture content plays an important role in biscuit production. This is because the moisture content has a direct impact on the quality of the biscuits produced. Increased moisture content in biscuits correlates with diminished shelf life and quality of the products. High moisture or water content in biscuit can increase the risk of microbial growth and accelerate lipid oxidation, which produces a rancid odor, thereby reducing product quality and consumer acceptance and shortening shelf life (Le *et al.* 2026) The tolerance of the water content in biscuits, as specified in SNI number 2973:2022, is only 5% (SNI 2022).

Table 1 shows that only F0 (4.97±0.05) met the moisture content threshold requirements set by the SNI. According to a previous study, the addition of FPH increased the moisture content of the resulting product (Yuniarti *et al.* 2025). The moisture content of biscuits can also be influenced by their temperature and baking time (Hidayah *et al.* 2023). However, a higher fat content can reduces moisture content because it can reduce moisture retention (Sharma *et al.* 2025).

Amino acids are the result of protein breakdown by digestive or protease enzymes into simpler and more easily digestible forms in the body. Amino acids comprise essential amino acids that the body cannot produce. Consequently, essential amino acids must be acquired through dietary sources, while non-essential amino acids can be produced by the human body.

FPH is an innovation developed to utilize bycatches fish and increase its selling value, thereby contributing to efforts to reduce fishery waste. Fish protein hydrolysates can be prepared in several ways. However, enzymatic hydrolysis is the most commonly used method, which utilizes proteolytic enzymes (such as papain, alcalase, and protamex) to hydrolyze proteins into smaller peptide chains. In addition to being high in amino acids, FPH contains several bioactive compounds, such as antioxidants, antimicrobial, anti-cancer, ACE inhibitors, and immunomodulators (Idowu *et al.* 2021).

Table 2 shows no trends in amino acid increase or decrease. This is because amino acid content is influenced by several factors, such as the the protein composition of the product, the concentration of the incorporated hydrolysate, and the thermal processing. An elevation in protein content can augment amino acid levels, as amino acids are the result of protein degradation into smaller components.

However, the highest amino acid content was observed in F3 compared to the other formulations (F0, F1, and F2). In addition, Table

Table 2. Analysis of amino acid profile in 100 g biscuit substitute FPH

Type of amino acid	Amino acid profile (mg/kg) (mean±SD)				*p*
	F0	F1	F2	F3	
Essential amino acids (mg/kg)					
Leucine*	5,996±15.3[a]	5,530±45[b]	6,314±42.7[c]	11,378±32.8[d]	0.000
Histidine*	2,606±4.7[a]	2,625±19.8[a]	2,760±18.2[b]	6,139±41.6[c]	0.000
Isoleucine*	2,671±33.5[a]	2,416±1.3[b]	3,067±2[c]	4,045±9.4[d]	0.000
Lysine*	2,922±7.7[a]	3,183±3.8[b]	3,284±24.3[c]	3,955±18.7[d]	0.000
Valine*	3,067±11.7[a]	2,841±6.1[b]	3,299±20.2[c]	4,721±38.2[d]	0.000
Phenylalanine*	4,233±9.7[a]	3,656±3[b]	4,613±37.2[c]	10,169±45.8[d]	0.000
Treonine*	2,641±9.4[a]	2,187±2.4[b]	2,773±24.5[c]	4,392±15.7[d]	0.000
Non-essential amino acids (mg/kg)					
Aspartic Acid*	5,861±11.1[a]	5,746±13.1[a]	5,784±53.1[a]	8,932±91.3[b]	0.000
Alanine*	3,448±144[a]	3,543±0.04[a]	3,496±31.6[a]	4,871±7.7[b]	0.000
Arginine**	1,842±2,13[a]	3,055±18[a]	3,511±31.3[a]	6,05±94[b]	0.046
Glycine*	4,364±2.5[a]	4,378±2.04[a]	4,454±39.2[a]	7,935±107[b]	0.000
Glutamate Acid*	1,991±58.4[a]	17,144±37.4[b]	17,920±180[c]	37,269±44.6[d]	0.000
Proline*	7,318±25.1[a]	6,765±14.1[b]	7,134±62.8[c]	17,502±28.1[d]	0.000
Serine*	4,134±3.7[a]	3,555±10.85[b]	4,144±37[a]	9,182±62.2[c]	0.000
Tyrosine*	1,826±2.1[a]	608±0.1[b]	1,907±15[c]	4,836±8.9[d]	0.000

Tested using One-Way ANOVA; mean±SD in the same row with different [a,b,c,d] indicates significant difference on further test; *Following post hoc test using Tukey HSD; ** Following post hoc test using Duncan; F0 (0% FPH), F1 (15% FPH), F2 (17% FPH), and F3 (20% FPH); FPH: Fish Protein Hydrolysate; SD: Standard Deviation

2 presents a comparison of each formulation that increased accordingly. The highest levels of aspartic acid, histidine, isoleucine, leucine, lysine, valine, and phenylalanine were observed in F3.

The amino acid value of FPH includes essential and non-essential amino acids. This suggests that the incorporation of FPH enhanced the amino acid content of the final product. The more abundant amino acid content in food product, the better its nutritional value, especially the appropriate amino acid contents. Additionally, the inclusion of FPH at specific concentrations can boost the features and sensory aspects of biscuit (Purwandari *et al.* 2025).

Based on several previous studies, the addition of FPH promoted better sensory acceptance. The Maillard reaction, which occurs during roasting, reduces the bitterness of FPH and enhances the caramel and umami notes (Honrado *et al.* 2023). Additionally, use of FPH can enhance texture of biscuits, as the resulting Maillard reaction can soften the texture. The addition of fish protein hydrolysate to biscuits can also change the microstructure of biscuits to become denser with fewer voids, so that the texture of the biscuits produced is not easily broken (Honrado *et al.* 2023; Sharma *et al.* 2025).

Therefore, greater the amino acid concentration of a food product will result in better protein absorption by the body. The use of FPH in biscuits can enhance the nutritional bioavailability the nutritional content of the product, particularly in terms of amino acid and protein content. Therefore, it can increase the nutritional requirements of children and meet their daily protein needs, as amino acids are more easily digested by the body (Puspita *et al.* 2023).

Essential Amino Acid Score (EAAS) is used to figure out whether a protein source provides the necessary EAA in adequate amounts to meet dietary requirements and helps in assessing the nutritional value of proteins (Wolfe *et al.* 2024). The content and amount of amino acid scores can also be utilized to determine quality of protein contained in foods (Matthews *et al.* 2025).

Information in Table 3 shows that amino acid scores indicate the highest overall EAAS is in F3. The higher the amino acid score, the more digestible the EAA content of the food and the higher the protein quality (Matthews *et al.* 2025). This result revealed that the EAAS of the biscuit was increased by adding FPH. The enzymatic hydrolysis process enhances the bioavailability of these amino acid and improves their nutritional value (Honrado *et al.* 2023).

Restrictive amino acid is the primary amino acid found in the smallest quantity proportion to the body's requirements standard. In general, Lysine is a most low amino acid in grains and is

Table 3. Measurement of essential amino acid score of biscuit as compared to WHO/FAO

Amino acid (mg/g)	EAA FAO/ WHO(2013)*	Biscuit EAA score			
		F0	F1	F2	F3
Histidine	20	0.13	0.13	0.14	0.31
Isoleucine	32	0.08	0.08	0.10	0.13
Leucine	66	0.09	0.08	0.10	0.17
Lysine	57	0.05	0.06	0.06	0.07
Phenylalanine+Tyrosine	52	0.12	0.082	0.13	0.29
Threonine	31	0.09	0.07	0.09	0.14
Valine	43	0.07	0.07	0.08	0.11
Limiting amino acid	-	Lysine	Lysine	Lysine	Lysine

*Suggested EAA FAO/WHO (2013) pattern of requirement for young children (6 months to 3 years); EAA: Essential Amino Acid; FAO; Food and Agriculture Organization; WHO; World Health Organization

abundant in meat proteins (Al Solami & Korish 2024). However, this study found that Lysine was a limiting amino acid. This is probably due to the product was exposed to high temperatures during processing or storage, causing a Maillard reaction that reduced the bioavailability of Lysine.

Protein digestibility refers to the proportion or percentage of protein that may be digested, absorbed, and utilized by the human body's digestive enzymes after consumption (Muller *et al.* 2025). Increased protein digestibility with addition of fish protein hydrolysate is possible because fish protein hydrolysate has shorter peptide chains, resulting in higher digestibility and rendering it appropriate for usage as a protein enhancer in various dietary categories (Nemati *et al.* 2024).

Based on Table 4, it is known that F3 has the highest protein digestibility (78.30%) compared to other formulations. Table 4 also shows an upward trend in protein digestibility with increasing amounts of FPH, ranging from 33.61% to 78.30%. According to research conducted by Hashem *et al.* (2023), It is stated that products with the addition of FPH from red-bellied pacu fish meat has higher and better protein digestibility, reaching 65.2 to 96%, because FPH protein is a protein that contains complete amino acids and has good protein digestibility.

Table 4. Measurenment of protein digestibility and PDCAAS in biscuit

Formuation	Protein digestibility (%) (Mean±SD)	PDCAAS (%)
F0	33.61±0.19[a]	1.7
F1	42.58±0.44[b]	2.4
F2	56.37±0.46[c]	3.8
F3	78.30±0.83[d]	5.4

Tested using One-Way ANOVA; mean±SD in the same row with different superscript indicates significant difference on further test. Following post hoc test using Tukey HSD; F0 (0% FPH), F1 (15% FPH), F2 (17% FPH), and F3 (20% FPH); PDCAAS: Protein Digestibility-Corrected Amino Acid Score; SD: Standard Deviation

This is also supported by Sandbakken *et al.* (2023), an increase in the amount of FPH added to the product will be exactly proportionate to an improvement in protein digestibility. A comparable result was reported by Charoensri *et al.* (2025) which states that protein hydrolysate from salmon carcasses offers high-quality protein that is easily digested by the body. High protein digestibility is very important for toddlers because during their growth period, as protein plays a crucial part in establishing the path of a child's development and growth.

Improving the digestibility of protein and amino acids in a product can increase the PDCAAS. Products with high PDCAAS levels indicate that they contain all essential amino acids and are more easily digested by the body (Novia *et al.* 2023). Furthermore, Table 4 shows that the PDCAAS value increases with the addition of FPH. The highest PDCAAS score was reported in F3 (5.4%).

The low PDCAAS level in biscuits can be caused by various reasons, which include low limiting amino acid levels and low digestibility of protein (Gaudichon 2024). Table 3 shows that the limiting amino acid in EAAS is lysine, with a score of 0.05 mg/g to 0.07 mg/g. In addition, the protein digestibility in Table 4 only ranges from 33.61% to 78.30%. This is what causes the low PDCAAS content in the biscuit products produced (ranging from only 1.7% to 5.4%).

Nevertheless, based on several studies that have been conducted and the results shown in Table 2 up to Table 4, it can be determined that

F3, which has considerably greater protein and amino acid composition, has excellent potential to boost protein digestibility and PDCAAS levels. The high quantity of significant amino acids such as leucine, arginine, and phenylalanine also aids the improvement in the overall quality of the protein (Sandbakken *et al.* 2023).

Thus, the addition of FPH to biscuit products not only increases protein content but also has the potential to improve protein bioavailability and digestibility, making FPH-substituted biscuits a promising alternative snack food to support protein adequacy in young children. Further research is recommended to evaluate protein digestibility in vitro and in vivo to corroborate these findings and identify the appropriate level of FPH substitution in terms of nutritional quality and sensory acceptance.

CONCLUSION

The result demonstrate that FPH substitute for biscuit significantly enhance protein (9.24±0.17–14.87±0.06), fat (13.45±0.06–15.6±0.09), and amino acid content. The higher the amount of FPH, the higher the EAAS, protein digestibility (33.61±0.19–56.37±0.46), and PDCAAS (1.7–3.8) of the other formulations. These findings indicate that F3 (20% FPH) is the most nutritionally enhanced snack, making it a suitable alternative for toddlers. The present findings establish a foundational basis for the development of protein-enriched staple foods as adjunctive nutritional interventions for vulnerable pediatric populations. However, this study is limited by the scope of sensory assessment and shelf life evaluation. Further research is needed to determine the levels of organoleptic properties as well as the optimal formulation of biscuit products.

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DECLARATION OF CONFLICT OF INTEREST

The authors state that they have no known competing financial interests or personal ties that could impact the work reported in this study.

DECLARATION OF GENERATIVE AI IN SCIENTIFIC WRITING

The authors state that they have avoided using generative AI technology in the writing, analysis, or preparation of this manuscript.

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