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Fortification of rice crisps with fish protein hydrolysate (*Decapterus sp.*) for iron deficiency prevention

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ABSTRACT

Iron deficiency remains one of the most widespread nutritional challenges globally, often addressed through dietary supplementation and food fortification. However, the limited bioavailability of iron in common food sources often hampers these efforts. Recent interest has grown in the use of protein hydrolysates to enhance iron absorption due to their abundance of low-molecular-weight peptides. This study explored the fortification of rice crisps with fish protein hydrolysate derived from *Decapterus sp*, aiming to improve the nutritional quality of a familiar snack and its potential to help reduce iron deficiency risk. The hydrolysate was characterized by its peptide molecular weight distribution, which showed a predominance of peptides under 15 kDa. Tyrosine emerged as the most abundant free amino acid, comprising 40.75% of the total amino acid content in the hydrolysate. The digestibility of the fish protein hydrolysate (DPH) was measured at 31.78%, with a protein concentration of 121 ppm and an iron-binding activity of 0.025%. Among all formulations, the sample containing 15% hydrolysate (F3) was most favored in sensory tests, balancing nutritional enrichment with consumer acceptability. These findings suggest that incorporating fish protein hydrolysates into popular food products may offer a promising, sustainable approach to combat iron deficiency, particularly among vulnerable populations such as children and women of reproductive age.

Contribution/Originality: This study demonstrates the potential of fish (*Decapterus sp*) protein hydrolysate as a bioavailable iron fortificant in rice crisps, integrating peptide characterization and sensory evaluation to offer a functional food-based strategy for mitigating iron deficiency in vulnerable populations.

1. INTRODUCTION

Decapterus sp. commonly known as scads, are small pelagic fish widely distributed throughout the Indo-Pacific region. Belonging to the mackerel family, these fish are considered low-fat and are characterized by a high protein-to-lipid ratio. Their nutritional value lies in their rich content of essential amino acids, which are crucial for human growth and health. According to Irianto and Soesilo (2007), *Decapterus sp* contains approximately 22% protein and

1% fat, yielding about 109 kilocalories per 100 grams. The species was selected for this study due to its high availability and accessibility, particularly in local Indonesian communities.

Data from the Fish Quarantine and Quality Control Agency (2018) indicate that the annual production of *Decapterus sp.* in Indonesia from 2015 to 2018 averaged 26,334.49 tons, with a 40.2% increase over that period. Despite its nutritional potential, fish consumption faces sensory challenges, particularly an unappealing odor that makes it less favorable compared to other protein sources such as meat, poultry, and eggs. To address this issue, processing techniques like steam blasting have been explored to reduce the fishy smell and enhance consumer acceptance. Nonetheless, fish remains a valuable source of both protein and iron. Mohanty et al. (2019) emphasized the critical role of fish as a primary dietary source of iron, particularly in regions where iron deficiency is common.

Iron deficiency remains a major global health concern, affecting an estimated two billion people, particularly women and children (Dias, Sanchez, Bartolo, & Oliveira, 2003). Other micronutrient deficiencies, such as vitamin A deficiency, which causes blindness in 2.8 million children under five years, and iodine deficiency disorders, which impact 740 million individuals, further emphasize the importance of addressing hidden hunger. One significant contributor to iron deficiency is the low bioavailability of dietary iron. Iron deficiency can lead to anemia and impaired physical and cognitive development (Tsakirpaloglou et al., 2023).

Strategies to combat this issue include supplementation and food fortification. Boonyaves, Wu, Gruissem, and Bhullar (2017) suggested the development of nutrient-dense fortified foods as a promising solution, although their efficacy is often hindered by poor micronutrient bioavailability. In this context, rice crisps were chosen as the food matrix due to their popularity, especially among children and adolescents, making them suitable vehicles for fortification.

This study explores the application of *Decapterus sp* protein hydrolysate (DPH) as a functional fortifying agent in rice crisps. It is hypothesized that the hydrolysate may facilitate iron absorption by forming peptide-iron complexes and enhance absorption. The physicochemical properties of the fortified rice crisps, including bulk density, viscosity, color, iron-binding capacity, and amino acid profile, were systematically evaluated. The integration of fish protein hydrolysates into familiar snack products offers a novel and potentially effective approach to combating iron deficiency anemia through functional food innovation.

2. MATERIALS AND METHODS

2.1. Materials

The primary ingredient used in this study was a protein hydrolysate derived from 100% Decapterus sp., a small pelagic fish widely available in Indonesia. The fish was sourced from Muara Angke, Jakarta. Additional ingredients used to produce the rice crisps included corn flour, rice flour, modified starch (Ingredion Thai Co., Ltd.), salt (Refina®), margarine (Blue Band®), and an emulsifier.

2.2. Preparation of Decapterus sp. Protein Hydrolysate (DPH)

The hydrolysate was prepared following a modified version of the method by Giarni et al. (2024). A total of 250 grams of filleted Decapterus sp. were steam-blasted, homogenized, and diluted in distilled water at a 1:2 (w/v) ratio. Bromelain enzyme was then added at a concentration of 1:1500 g/g of fish mass, and the mixture was incubated at 60°C for 2 hours. Enzymatic activity was halted by heating the mixture to 80–90°C for 15 minutes. The resulting hydrolysate was dehydrated at 45°C for 24 hours and then ground into a powder form.

2.3. Rice Crisp Formulation and Processing

Four rice crisp formulations were developed with varying concentrations of DPH: 5% (F1), 10% (F2), 15% (F3), and 20% (F4). The formulation is shown in Table 1.

Table 1. Rice crisp formulations (% w/w).

Ingredients (%)	F1	F2	F3	F4
Corn flour	75	70	65	60
HPI	5	10	15	20
Rice flour	14	14	14	14
Modified starch	2	2	2	2
Salt	0.5	0.5	0.5	0.5
Butter	2	2	2	2
Emulsifier	1.5	1.5	1.5	1.5

The production steps included mixing, steaming, extrusion, drying, soaking, and fluidization. Ingredients were mixed thoroughly and steamed for 30 minutes. The mixture was extruded using a single-screw extruder (Giant Machine Extrude 1100 W, 60 rpm). Dried at 45°C for 24 hours, the extrudates were then soaked in water at a 1:1 ratio and fluidized (Giant Machine Fluidized 1100 W) at 120°C for 10 minutes. The resulting crisps were subjected to physicochemical and sensory analysis.

2.4. Iron-Binding Activity of DPH

Iron-binding activity of the hydrolysate was determined using the phenanthroline method (Giarni et al., 2024). One gram of DPH was dissolved in 50 mL of distilled water, shaken for 1 hour, filtered, and reacted with $FeSO_4$ in phosphate buffer. The resulting solution was reacted with hydroxylamine hydrochloride, acetate buffer, and phenanthroline, incubated for 20 minutes, and measured at 510 nm. Iron concentration was calculated using a standard curve.

2.5. Protein Digestibility of DPH

In vitro protein digestibility was assessed using a modified version of the method by Saputra (2014). A 0.5 g DPH sample was dissolved in distilled water (pH 8.0), treated with and without enzyme, incubated at 37°C, precipitated with 0.1 M TCA, and centrifuged. The supernatant was analyzed using the Folin reagent, and absorbance was measured at 578 nm.

2.6. Amino Acid Composition of DPH

Free amino acid content was quantified by HPLC with pre-column derivatization using ortho-phthalaldehyde (OPA), forming detectable fluorescent derivatives. The analysis was conducted following the method described by Giarni (2024).

2.7. Molecular Weight Analysis

The molecular weight profile of the hydrolysate was determined using SDS–PAGE. A 1 g sample was dissolved in Tris-HCl buffer (pH 8.5), vortexed, centrifuged, and loaded onto a gel composed of 5% stacking and 15% separating gels. Electrophoresis was performed at 140–160 V. Protein bands were visualized using Coomassie or silver stain, then destained with methanol-acetic acid-water solution.

2.8. Bulk Density of Rice Crisp Formula

Bulk density of the rice crisp formulations was measured using a 50 mL graduated cylinder following the procedure by Muchtadi and Sugiyono (1992). The difference between the full and empty cylinder weights was divided by the volume to obtain bulk density (g/mL).

2.9. Viscosity of Rice Crisp Formula

Pasting properties were analyzed using a Rapid Visco Analyzer (RVA, Perten Instruments, Model 4500) with the STD1 profile, according to AACC Method 76-21.01 and ICC Standard No. 162. A 3.5 g starch sample was dispersed in 25 mL of water, and various viscosity parameters were recorded.

2.10. Color Measurement of Rice Crisp Formula

Color was measured using a dual-beam reflectance spectrophotometer (AGERA, HunterLab). The results were recorded as CIE L*, a*, b* values, with gloss measurements performed using a 51 mm port size. The analysis followed ASTM D523 and ISO 2813 guidelines.

2.11. Sensory Evaluation

The hedonic evaluation was conducted using a nine-point preference scale. Sensory analysis focused on taste as the primary attribute, aiming to identify which Rice Crisp formulation was most preferred.

A nine-point hedonic test was conducted with 31 semi-trained panelists from BRIN's Agroindustry Laboratory (LAPTIAB) at BRIN-KST BJ Habibie Serpong, following the method described by Yuniarti and Tunggal (2015). Each rice crisp formulation was evaluated for color, flavor, texture, taste, and overall acceptability. Samples were coded with random numbers, and tea was provided between tastings to neutralize the palate. The scoring criteria used by the panelists are detailed in Table 2.

Table 2. Organoleptic test and hedonic test assessment score.

Score	Criteria
1	Dislike extremely
2	Dislike very much
3	Moderately like
4	Slightly dislike
5	Normal/Neutral
6	Like slightly
7	Like Moderately
8	Like very much
9	Like extremely

2.12. Statistical Analysis

Data were analyzed using SPSS v23.0 for Windows. Non-parametric tests (Kruskal-Wallis and Mann-Whitney U) were applied, and a significance threshold of $p < 0.05$ was used.

3. RESULTS AND DISCUSSION

3.1. Iron-Binding Capacity of DPH

The protein hydrolysate derived from *Decapterus sp* (DPH) exhibited an iron concentration of 121 ppm and an iron-binding value of 3.057 ppm, which is equivalent to a binding activity of 0.025% (Table 3). Although relatively modest, this finding aligns with previous reports such as Chunkao, Youravong, Yupanqui, Alashi, and Aluko (2020) who reported iron-binding activities of 0.036% in mung bean hydrolysates. Similarly, Gaviria et al. (2024) also reported comparable activity in hydrolysates derived from California Red Worm. Iron-binding peptides often result from enzymatic hydrolysis, where protein chains are broken into smaller peptides with exposed functional groups (Zhang et al., 2021), enhancing metal chelation potential. This suggests that DPH could serve as a natural iron-enhancing agent in food matrices.

Table 3. Nutritional content of DPH.

Sample	**Protein content** ppm	**Iron binding** ppm	**Iron Binding Activity** %	**Protein Digestibility** %
Control	34.161	2.521	0.033	-
DPH	121.000	33.315	0.025	31.776

3.2. Protein Digestibility of DPH

The digestibility of *Decapterus* protein hydrolysate was 31.78%, as a result of the combined effects of the steam-blasting process and enzymatic hydrolysis. These processes break down protein into smaller, more absorbable peptides and denatured structures, improving enzymatic access. While this figure is lower than those reported for cod or salmon hydrolysates (often exceeding 90%) (Bechtel & and Johnson, 2004), it is consistent with findings in herring hydrolysates (Šližytė, Carvajal, Mozuraityte, Aursand, & Storrø, 2014). Residual anti-nutritional components or interactions within the matrix may account for the moderate digestibility. Nevertheless, the results are promising, as the treatment still significantly enhances the protein's nutritional accessibility, indicating the potential of DPH for functional food applications.

3.3. Amino Acid Composition of DPH

The amino acid analysis revealed that DPH contained 41.55% free amino acids, markedly higher than the control sample's 9.27%, as seen in Table 4. Among them, tyrosine was dominant, comprising 40.75% of the total content. Increases were also noted in glycine, histidine, alanine, and cysteine. This enhanced profile is a direct consequence of enzymatic hydrolysis, which cleaves peptide bonds and liberates individual amino acids. A diverse amino acid composition contributes to functional properties such as antioxidant potential and mineral chelation, as noted by Gotti et al. (2022), making DPH a nutritionally enriched ingredient for food fortification.

Table 4. Amino acid profile of DPH (%).

Type of Amino Acids	Time retention	Control (%)	DPH (%)
Aspartic acid	1.787	0.062	0.347
Glutamate	1.855	Not detected	Not detected
Serine	2.742	0.001	0.004
Glycine	3.019	0.05187	0.249
Leucine	3.431	Not detected	Not detected
Histidine	3.577	0.00694	0.115
Threonine	3.966	0.001	0.004
Arginine	4.358	0.001	0.004
Proline	5.889	Not detected	Not detected
Alanine	6.079	0.009	0.057
Cysteine	6.803	Not detected	0.001
Tyrosine	7.526	9.124	40.749
Valine	7.904	Not detected	Not detected
Methionine	9.179	0.003	0.017
Isoleusin	9.401	0.001	0.004
Leusin	9.957	0.004	0.002
Phenylalanine	13.793	0.007	0.001
Total		9.270	41.554

3.4. Molecular Weight Distribution of DPH

SDS–PAGE results (Figure 1) showed that peptides in DPH predominantly had molecular weights below 15 kDa. This low molecular weight range is consistent with the bioactive fraction of hydrolysates from other marine sources (Borges et al., 2023). The steam blasting technique, combined with enzymatic hydrolysis, facilitates the release of small peptides that are not only more digestible but also functionally active. According to Beraldo, Nogueira, Prata, and Grosso (2021) and Anggraini and Yunianta (2014), such peptides tend to exhibit improved bioavailability and metal-binding properties, supporting the functional design of iron-enhanced foods.

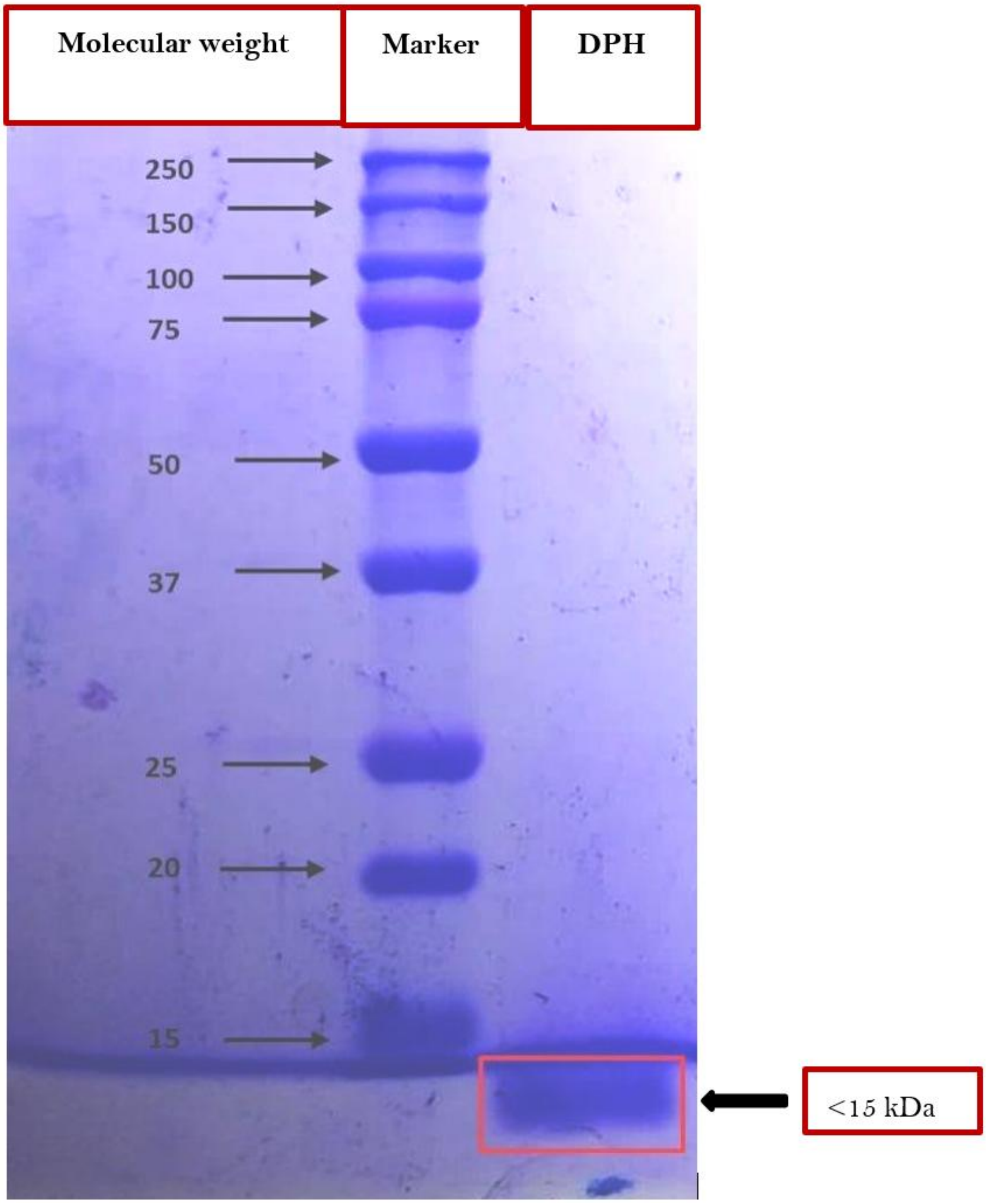

Figure 1. Molecular weight of protein of DPH.

3.5. Bulk Density of Rice Crisp

The rice crisp formulations showed varying bulk densities, with F1 (5% DPH) recording the highest (0.5421 g/mL) and F3 (15% DPH) the lowest (0.4775 g/mL) (Figure 2). This inverse relationship suggests that increasing DPH levels reduces the compactness and increases porosity in the crisps. This may be attributed to protein content altering the matrix structure. Winarno (2021) and Miura, Ito, and Miyoshi (2023) have demonstrated that higher protein or fiber content can create more porous structures, reducing bulk density in extruded products. Additionally, corn flour, a primary component in the formulations, contributes to increased void fraction, forming larger air pockets.

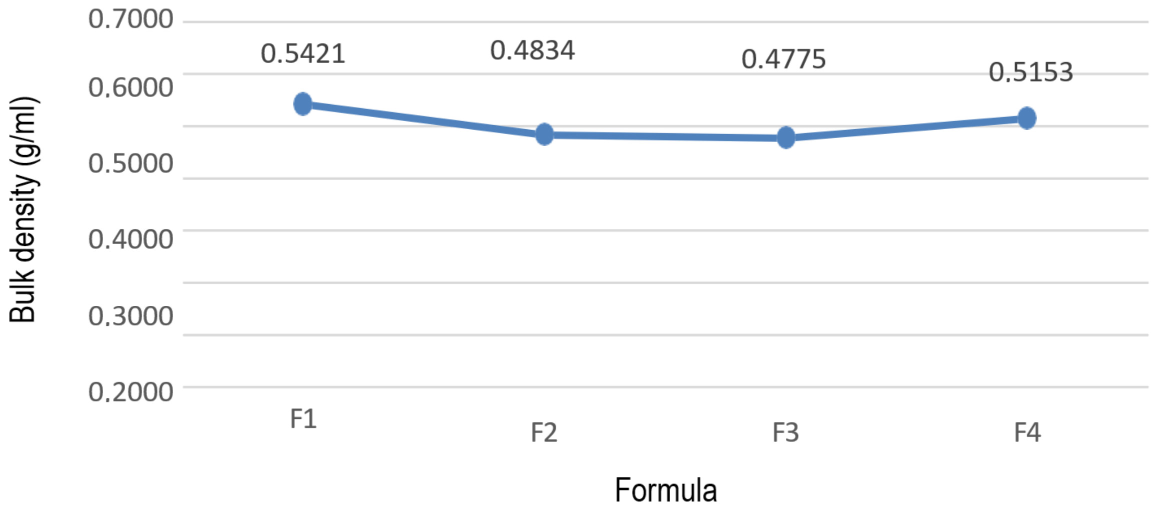

Figure 2. Bulk density of rice crisp.

3.6. Viscosity

Peak and breakdown viscosities were highest in F1 (Table 5), which contained the most starch and least hydrolysate. These values indicate the maximum swelling capacity and stability of starch granules. As DPH levels increased (F2–F4), the viscosities decreased, likely due to the dilution of starch and interaction of peptides with the gelatinization process. The final viscosity, which reflects paste consistency after cooling, was higher in F1 but gradually increased again in higher hydrolysate formulations. Fitriani, Yusmarini, Riftyan, Saputra, and Rohmah (2023) suggested that moisture control and extrusion temperature can influence such properties, supporting this trend.

Table 5. Physicochemical analysis.

Sample	Color			Density	Viscosity (cP)			
	L*	a*	b*	g/ml	Final viscosity	Set back	Peak viscosity	Breakdown
F1	53.34	9.69	34.73	0.5421	1202	66	3258	2122
F2	54.47	10.56	35.76	0.4834	461	220	260	19
F3	51.25	10.27	32.18	0.4775	635	360	296	21
F4	46.21	11.13	30.87	0.5153	758	185	730	157

Note: (*) refers to CIELAB color space.

3.7. Color Analysis of Rice Crisp

Color analysis indicated that higher DPH concentrations reduced brightness (L* value) and intensified red (a*) and yellow (b*) hues (Table 5). F2 showed the brightest appearance (L* = 54.47), while F4 appeared darkest (L* = 46.21). These changes are likely due to the Maillard reaction, where amino acids react with reducing sugars during processing, forming darker compounds. Similar effects have been documented by Giarni et al. (2024) and Jeyakumari, Janarthanan, Chouksey, and Venkateshwarlu (2016) in hydrolysate-enriched products. The increasing a* and b* values correspond to a more reddish-yellow tone, influenced by the hydrolysate's natural pigmentation.

The brown color and increasing amount of hydrolysate used led to a decrease in the brightness of the rice crispy. According to the literature, the brown color and increasing amount of hydrolysate used lead to a decrease in the brightness of the rice crispy (Jeyakumari et al., 2016). The analysis results show a lower value, whereas a higher b value indicates that the samples exhibit a bright yellow color. The results are shown in Figure 3.

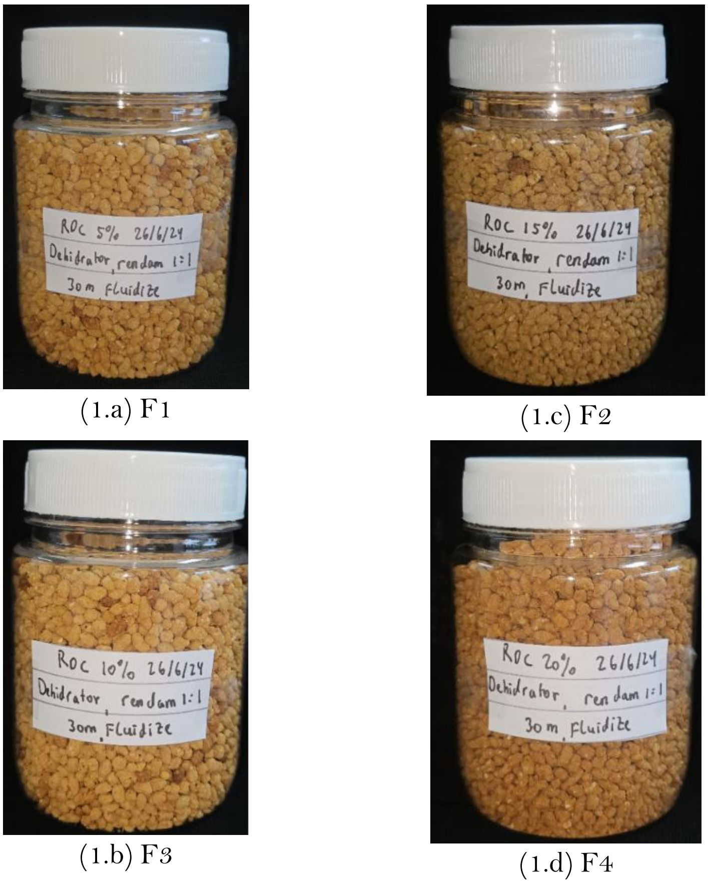

Figure 3 rice crisp formulation with variable DPH content (1.a) 5%, (1.b) 10%, (1.c) 15%, (1.d) 15%.

3.8. Sensory Evaluation Discussion

The sensory evaluation results for the four Rice Crisp formulations (F1–F4) are summarized in Table 6. Among them, formulation F3 consistently received the highest scores across most sensory attributes, indicating a stronger overall preference by the panelists compared to the other formulations. In terms of color, F3 recorded the highest mean score (6.19), classified as slightly liked, suggesting a more visually appealing appearance. This was followed closely by F4 (6.00) and F1 (5.87), while F2 (5.71) remained within the same preference category, indicating that all formulations were generally acceptable in terms of appearance.

For aroma, F3 again achieved the highest score (5.19), though it remained within the neutral preference category. F2 (4.77) and F1 (4.39) received slightly lower ratings, while F4 obtained the lowest score (4.29), placing it in the slightly disliked range. These findings suggest that aroma was a less favorable attribute overall, potentially due to the presence of fish protein hydrolysate, which may have contributed to off-odors.

Table 6. Hedonic test results of rice crisp with different formulations.

Parameter	Formula	Score mean		Sig.
Colour	F1	Neutral	5.87 ± 1.203^{a}	0.05
	F2	Neutral	5.71 ± 1.270^{a}	
	F3	Like slightly	6.19 ± 1.077^{a}	
	F4	Like slightly	6.00 ± 1.095^{a}	
Flavour	F1	Slightly dislike	4.39 ± 1.687^{ab}	
	F2	Slightly dislike	4.77 ± 1.359^{ab}	
	F3	Neutral	5.19 ± 1.621^{b}	
	F4	Slightly dislike	4.29 ± 1.657^{a}	
Texture	F1	Neutral	5.58 ± 1.455^{a}	
	F2	Neutral	5.77 ± 1.407^{ab}	
	F3	Like slightly	6.29 ± 1.160^{b}	

Parameter	Formula	Score mean		Sig.
	F4	Neutral	5.52 ± 1.546^{a}	
Taste	F1	Slightly dislike	4.32 ± 1.759^{a}	
	F2	Slightly dislike	4.87 ± 1.408^{ab}	
	F3	Neutral	5.68 ± 1.661^{b}	
	F4	Slightly dislike	4.87 ± 1.628^{ab}	
Overall taste	F1	Slightly dislike	4.61 ± 1.498^{a}	
	F2	Slightly dislike	4.94 ± 1.315^{a}	
	F3	Neutral	5.71 ± 1.510^{b}	
	F4	Slightly dislike	4.90 ± 1.326^{a}	

Note: Result are mean±standard deviation: Mean at the same parameter with different superscript indicates significantly different ($P<0.05$). The letter 'a' indicates that the same parameter is not significantly different from other values labeled 'a', whereas the letter 'b' indicates a significant difference from those labeled 'a'.

The texture of F3 was rated most favorably, with a mean score of 6.29 (Slightly liked), indicating a desirable crispness and mouthfeel. F2 (5.77) and F4 (5.52) were rated as neutral, while F1 (5.58) also fell within the neutral range, albeit slightly higher. The superior texture score of F3 may be attributed to its better expansion properties or a more uniform crisp structure.

Regarding taste, F3 again outperformed the other samples, receiving a score of 5.68, which falls within the neutral category but was notably higher than the rest. F2 and F4 shared the same score (4.87), while F1 received the lowest rating (4.32, slightly disliked). The lower taste score for F1 may reflect an unbalanced seasoning profile or an overpowering fishy flavor from the hydrolysate.

For overall acceptance, F3 garnered the highest rating (5.71), indicating that it was generally well-received by the panelists. The other formulations, F1 (4.61), F2 (4.94), and F4 (4.90), received neutral or slightly lower ratings, suggesting they were moderately acceptable but less preferred compared to F3.

In summary, among the four formulations, F3 (15% DPH) consistently received the highest scores across key sensory attributes: color, flavor, texture, and overall acceptability. Although the aroma was not significantly preferred across samples, taste and texture were notably better in F3. Panelists appreciated the balanced flavor and crispiness, likely a result of optimal hydrolysate integration. While F1 and F2 had acceptable sensory profiles, F4 (20% DPH) tended to receive slightly lower ratings, possibly due to stronger fishy notes or textural imbalances. While aroma and taste were rated less favorably across all samples, these attributes could potentially be improved through formulation adjustments, particularly by enhancing flavor masking and aroma control to better balance the sensory profile of fish protein hydrolysate-based rice crisp products.

3.9. Practical Implications and Perspectives

The incorporation of fish protein hydrolysate (DPH) into rice crisps not only offers a promising strategy to improve iron availability but also aligns with the sustainable utilization of marine resources. By valorizing underutilized species such as *Decapterus sp.*, this approach supports circular economy principles within the food industry. Moreover, the development of nutrient-enriched snack products is particularly relevant for addressing micronutrient deficiencies among vulnerable groups, such as school-aged children and women of reproductive age, who are more susceptible to iron deficiency anemia.

Despite these benefits, the incorporation of fish-based hydrolysates into commonly consumed products such as rice crisps also raises important considerations regarding consumer perception. The potential for off-flavors or undesirable odors, even at low inclusion levels, must be addressed through further sensory optimization and possibly flavor-masking techniques. Additionally, regulatory and labeling aspects regarding the use of marine-derived ingredients in fortified snacks require attention to ensure compliance and market acceptance.

Future studies should also investigate the synergistic effects of DPH when combined with other micronutrients (e.g., vitamin C or zinc), as well as its long-term stability and efficacy under real storage and consumption conditions.

3.10. Study Limitation

Despite promising findings, this study has several limitations. First, the in vitro evaluation of iron-binding capacity and protein digestibility may not fully represent actual bioavailability in human subjects. Further in vivo studies are necessary to confirm the physiological efficacy of DPH-fortified rice crisps. Additionally, while sensory testing indicated a preference for the 15% DPH formulation, the sample size and demographic diversity of the sensory panel were limited. Expanding the sensory evaluation to include broader age groups and consumer profiles could provide more comprehensive insights into product acceptance.

4. CONCLUSION

This study demonstrated that fortification of rice crisps with *Decapterus sp.* protein hydrolysate (DPH) enhances their nutritional and functional properties, particularly with respect to iron-binding activity and amino acid content. The 15% DPH formulation (F3) was most preferred based on sensory evaluation and offered a balance between nutritional improvement and product acceptability. The hydrolysate, rich in low-molecular-weight peptides and free amino acids (notably tyrosine), showed potential for improving iron bioavailability. These findings suggest that fish protein hydrolysates such as DPH can serve as effective functional ingredients for developing iron-enriched food products to combat iron deficiency, especially in vulnerable populations.

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Competing Interests: The authors declare that they have no competing interests.
Authors' Contributions: All authors contributed equally to the conception and design of the study. All authors have read and agreed to the published version of the manuscript.

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