Protein enrichment in instant pudding with fish protein hydrolysate (FPH) from trash fish

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Abstract. Fish Protein Hydrolysate (FPH) from trash fish is a product derived from fish through the process of protein hydrolysis, into smaller protein, including peptides and eventually amino acids. The FPH is used to enhance the characteristics of various food products, such as to amino acid profiles, and improve nutritional content. The purpose of this study was to increase protein levels and flavor intensity profiling instant pudding by adding FPH. The design experimental utilized the Complete Random Design (RAL) method. The study evaluated several parameters related to pudding quality: Hedonic Quality, Taste Intensity and Proximate, used ANOVA (Analysis of Variance) to compare means among different treatments (pudding with varying levels of FPH). The protein content of instant pudding ranged between 7.68-8.77%, higher than comercial instan pudding. The protein content of ready-to-eat pudding ranged from 1.84-2.28%, The highest acceptance rate observed was 5% for pudding with added FPH. Taste intensity tests revealed that adding FPH at higher concentrations resulted in intensified burning, fishy, and bitter aromas. The highest protein content was achieved with the addition of 15% FPH. The study provides valuable insights into how FPH impacts instant pudding.

1 Introduction

The Blue Economy concept, launched by the Ministry of Marine Affairs and Fisheries, can create additional economic value from non-economic products of fisheries and business activities that do not have a negative impact on the environment. The principle of the blue economy is the efficient use of nature's raw materials from marine so that there is no waste, which can have a negative impact on the environment [1]. This is a challenge for the fishing industry, which produces 30-40% of the waste or by-products it processes [2]. Another fishery by-product that is not the main target of fishermen is trash fish, which is caught in nets during the fishing process. Trash fish is often described as fish that is unfit for human

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consumption due to the unhygienic handling process. This makes trash fish a low economic fishery product [3].

Trash fish is biotechnologically processed as a good source of amino acids in FPH and is more economical. This makes trash fish a raw material that can be used to produce high value products [4]. The FPH is a source of amino acids that have biological activity resulting from hydrolysis between proteins with acids or strong alkalis and proteolytic enzymes, resulting in the release of amino acids that make up protein molecules by breaking the peptide bonds in proteins to form amino acid [5]. Using FPH has the potential to improve the quality of food ingredients using its functional properties including high protein, amino acids, high solubility and bioactive compounds. The application of FPH to food is through enrichment. This results in innovative food products that are high in protein. Intentionally increasing the nutrient content of food is called enrichment [6].

Enrichment is a nutritional intervention to combat micronutrient deficiencies and reduce the incidence of undernutrition or can be interpreted as nutrient enrichment of food products [7]. Enrichment aims to create products with rich nutritional content [8]. Enrichment of FPH can be done in sweet products with the best concentration of 15% which produces the highest protein content [9]. Pudding contains no less than 1.4% protein content [10]. It is understood by 86% of people who recognize the benefits of pudding, and 100% are capable of making it yet approximately 14% remain unaware of its benefits. Due to a preference for convenience and the quick process involved, technological advancements have led to the production of instant pudding in powder form. This allows for extended storage and reduced spoilage, attributed to its low moisture content of about 2.70% [12]. Given the relatively low protein content in pudding, the use of FPH is proposed to enhance its protein levels. The research aimed to increase the pudding's protein content in a manner acceptable to the panelists.

2 Materials and Methods

2.1 Materials

The ingredients used in making pudding consisted of FPH rucah from the hydrolysis of rucah fish using the method [13] from the AUP Polytechnic Postgraduate Laboratory, skimmed milk (Nestle), powdered sugar (Bola Deli), vanilla (Koepoe-koepoe), agar flour (Rucira), konjac flour (Galicgum), manga flour (Rainpow), mango flavour, natural colouring (Saffron), retort pouch packaging and 35 mL plastic cups. Chemical analysis materials used to perform pro-analysist grade from Merck (Singapore) included concentrated H_2SO_4, K_2SO_4, dan $CuSO_4$ (3:1), NaOH 40%, H_3BO_3 4% dan 2%, metil red indicator (MM), blue cresol green indicator (BCG), bromocresol green indicator 0.1%, metil red 0.1%, alcohol 95%, HCl 0.1 N, borax solution 0.1 N, ethanol 96%, distilled water, and N-Hexane.

2.2 Equipments

Tools for making pudding include digital scales, spoons, measuring cups, gas stoves, and bowls, mixers, and plastic cups. Chemical analysis equipment includes analytical balance, spatula, measuring cup, beaker glass, desiccator, oven, volumetric pipette, filter paper, funnel, 1 set of Kjeltec, digestor, protein tube, 100 mL and 500 mL volumetric flask, 100 mL measuring cup, 10 mL goiter pipette, drop pipette, tissue, soxtec system, fat sheath, lumping, ectraction cup, porcelain cup, soxtec furnace, hot plate, tongs, stainless steel spoon, buchner funnel.

2.3 Experiment Design

The processing procedure, a key part of our research, is carried out with precision. It involves making pudding, a method that has been modified from the research of [14]. Our research method, which uses a Completely Randomized Design (CRD), is based on the analysis of the effect of adding FPH, as per the research by Asare et al., (2018). Three treatments, one control (commercial pudding) consisting of F1 (FPH 0%), F2 (FPH 5%), F3 (FPH 10%), and F4 (FPH 15%) with three repetitions. The instant pudding and ready-to-eat pudding, products of our meticulous research, are subjected to a comprehensive analysis. This includes moisture content, ash content, fat content, protein content, crude fiber, carbohydrate content [15]. Organoleptic intensity test (burning aroma, fishy aroma, mango aroma, milk aroma, bitter taste, salty taste, sweet taste, mango taste, milk taste, filth, color intensity, chewy texture and soft), hedonic quality (color, aroma, taste and texture), and overall hedonic tested by 30 panelists [16] .

2.4 Data analysis

The data obtained were analyzed using ANOVA variance analysis to determine the effect of adding FPH to pudding. A further Tukey test was carried out if the study results were significantly different—data analysis for organoleptic test results using non-parametric statistical tests, namely Kruskal-Wallis and Mann Whitney.

3. Result and Discussion

3.1 Chemical composition of instant pudding

The results of the chemical composition of instant pudding can be seen in Table 1.

Table 1 The chemical composition of instant pudding

Composition content	Comersial Pudding	F1	F2	F3	F4
Moisture (%)	0.69±0.73^{c}	2.66±0.093^{a}	2.96±0.14^{ab}	2.91±0.33^{ab}	3.28±0.30^{b}
Ash (%)	1.97±0.01	4.84±0.03	4.61±0.15	4.77±0.04	4.29±0.02
Lipid (%)	0.45±0.24	0.41±0.11^{ab}	0.39±0.01^{ab}	0.30±0.19^{a}	0.67±0.44^{b}
Protein (%)	0.83±0.02	7.68±0.41^{b}	8.46±0.58^{bc}	8.65±0.15^{bc}	8.77±0.12^{c}
Crude fiber (%)	5.19±0.01	30.00±0.54^{d}	28.43±0.08^{d}	21.35±0.56^{c}	10.83±0.14^{b}
Carbohydrate (%)	90.86±0.21	54.41±0.40^{a}	55.14±0.52^{a}	62.02±1.01^{b}	72.71±0.66^{c}

Values with different superscript letters in the column indicate significant differences between treatments ($p<0.05$).

Adding FPH with different concentrations significantly affected the moisture content value of instant pudding ($P<0.05$). The 15% FPH (F4) treatment has quite a high moisture content compared to the others due to the ability of the hydrolyzate to bind water by absorbing moisture from the environment, and the addition of enzyme ingredients to FPH increases dissolved protein, resulting in increased hydration of moisture content [17] Tukey's further test showed that the addition of FPH was not significantly different between treatments. This is because inorganic substances result from material composition and processing methods, including the time and temperature used. High ash content can be caused by mixing alkaline and acidic compounds during hydrolysis [18]. The addition of FPH was significantly different from the fat content of instant pudding ($p<0.05$).

The fat content value of instant pudding ranges from 0.41-0.67%; the low fat in the pudding is due to the use of FPH, which has a low-fat content due to the hydrolysis, extraction or filtration process, which can cause the membrane structure to be released or damage to the lipid membrane [19], water-insoluble precipitates and mixes in the residue layer, there is a reduction in fat content and compound binding ability because the presence of hydrophobic and hydrophilic peptides influences the binding of fat in fish so that it becomes a hydrolysate product [4]. The protein content of instant pudding ranged between 7.68-8.77%; the addition of FPH significantly differed from the protein content of instant pudding ($p<0.05$).

The protein content value tends to increase with the addition of FPH, thus providing a significant difference. Increasing the amount of protein indicates an increase in total nitrogen (and changes in water-insoluble properties change to water-soluble nitrogen compounds and simple compounds such as peptides and amino acids [18]. FPH is hydrolyzed into simpler amino acids using acids and enzymatically, so the absorption process is faster than larger proteins [20]. The crude fiber content produced ranged from 10.83-30.00%; the addition of FPH significantly differed from the protein content of instant pudding ($p<0.05$).

The value of crude fiber content decreased along with the addition of FPH. This is because the amino acids contained in FPH do not directly affect the crude fiber content. After all, the high crude fiber content is caused by the agar ingredient from *Gracilaria* seaweed, which contains relatively high levels of polysaccharides [21]. FPH contains a small amount of glycogen, including glucose, fructose, sucrose, monosaccharides, and disaccharides [18]. The carbohydrate content of instant pudding, ranging between 54.51-72.71%, saw a significant increase with the addition of FPH ($p<0.05$). This increase in carbohydrate levels, influenced by the fiber content, is a key finding. The crude fiber, containing polysaccharides, plays a role in this increase. Polysaccharides, complex carbohydrates consisting of maltodextrin, are a significant component of FPH powder, contributing to the rise in carbohydrate levels [22].

3.2 The chemical composition of ready-to-eat pudding

Chemical testing is carried out to analyze, identify, and measure the chemical composition of food. Moisture content decreased along with increasing FPH concentration. The decrease in moisture content occurs due to increased water during the cooking process [14]. The colloidal nature of protein is that peptide bonds absorb water and swell, decreasing moisture content [23]. Water binding occurs due to the action of enzymes during the cooking process [24] and the presence of maltodextrin in the FPH content, which was deliberately added during hydrolysis [25]. Suppose the hydrolyzate is added to the pudding. In that case, the amino acids will absorb and bind the water added to the processing process, thus increasing the solubility of the protein, which causes a decrease in the moisture content.

The results of the One Way ANOVA test with a confidence level of 95% showed that the addition of FPH significantly differed from the moisture content of instant pudding ($p<0.05$) (Table 2). Tukey's further test showed that the moisture content significantly differed between one treatment and another. The moisture content of ready-to-eat pudding ranges from 73.92-76.07%. The ash content of ready-to-eat puddings ranged from 0.76-0.92%; the addition of FPH to ready-to-eat puddings was significantly different ($p<0.05$). Adding water and cooking at high temperatures for a long time causes mineral degradation, resulting in low ash content in the pudding [14]. Moisture content influences ash content [26]. The ash content in food comes from the inorganic ingredients from it [19].

The results of the chemical composition of ready-to-eat pudding can be seen in Table 2.

Table 2 Chemical composition of ready to eat pudding

Composition content	**Commersial pudding**	**F1**	**F2**	**F3**	**F4**
Moisture (%)	70.02±0.20[a]	76.07±0.72[d]	74.87±0.04[c]	74.25±0.12[bc]	73.92±0.34[b]
Ash (%)	0.38±0.13[a]	0.85±0.09[bc]	0.76±0.01[b]	0.92±0.02[c]	0.83±0.04[bc]
Lipid (%)	0.33±0.06[a]	0.28±0.07[a]	0.36±0.08[a]	0.59±0.02[a]	0.45±0.05[a]
Protein (%)	0.21±0.02[a]	1.84±0.06[b]	1.93±0.02[b]	2.05±0.08[bc]	2.28±0.05[c]
Crude fiber (%)	3.78±0.20[a]	7.21±0.28[bc]	7.77±0.12[c]	6.60±0.55[b]	7.23±0.40[bc]
Carbohydrate (%)	25.29±0.18[c]	13.76±0.93[a]	14.31±0.20[ab]	15.59±0.47[b]	15.29±0.20[b]

Values with different superscript letters in the column indicate significant differences between treatments ($p<0.05$).

The fat content of ready-to-eat pudding ranges from 0.28-0.59%. The addition of FPH was not significantly different ($p>0.05$). The fat content value shows that adding FPH does not affect ready-to-eat pudding. Low-fat content is caused by low fat in FPH. Low-fat FPH undergoes a fat and protein separation process, which can reduce the fat content so that it does not affect the final product [27]. Low fat content can also be caused by the fat-binding ability, influenced by hydrophobic peptides and the amino acid charge contained in FPH [4]. The low fat content is due to the use of skim milk and other ingredients that have low-fat content [28]. The protein content of instant pudding ranged from 1.84-2.28%; the addition of FPH significantly differed from the protein content value of ready-to-eat instant pudding ($p<0.05$).

The protein content value increased with the addition of FPH. FPH causes the increase in protein content and concentration through protein hydrolysis into simple peptide bonds, namely amino acids [29]. The high protein content in FPH is due to the addition of enzymes during hydrolysis [17]. Hydrolysis reactions generally occur where ATP + H_2O becomes ADP + Pi + energy [13]. There is a conversion of insoluble proteins into soluble nitrogen compounds, and there are simpler compounds, namely peptides and proteins, which cause an increase in protein along with the addition of FPH [30]. The crude fiber content of ready-to-eat pudding ranges from 6.20-7.77%; the addition of FPH shows a significant difference. The addition of FPH did not affect the crude fiber content produced. The low crude fiber content of ready-to-eat pudding is due to processing by adding water and the cooking process, which produces stiff crude fiber and the presence of cellulose, which can cause it to be smaller than the crude fiber content of instant pudding [31].

3.3 Organoleptic results of pudding intensity

Organoleptic testing is carried out to evaluate the organoleptic properties of a product by assessing the intensity of several aroma parameters (burnt, fishy, fruity, milky), taste (bitter, salty, sweet, fruity, milky), appearance (filth), color, and texture (chewy and soft). Intensity testing results provide information about several attributes' strengths in the product [32]. The results of the taste intensity of ready-to-eat pudding can be seen in Figure 1.

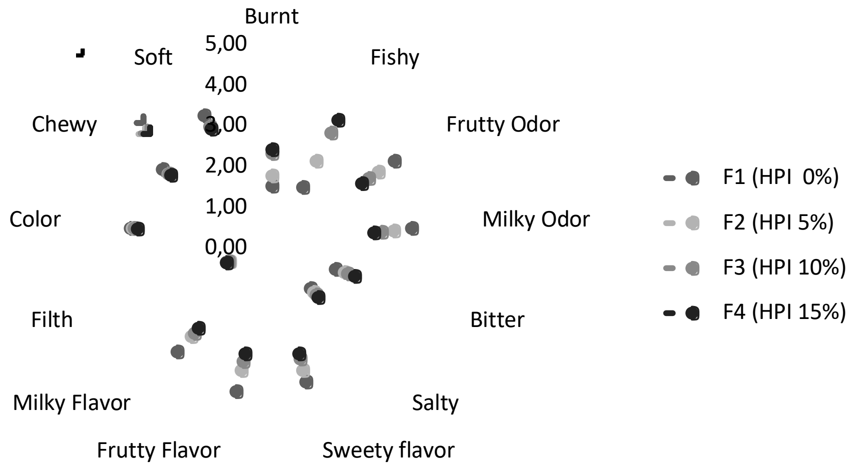

Fig. 1. Ready-to-eat pudding flavor intensity.

The results of the Kruskal-Wallis statistical test have a significant value of p=<0.001 for the burning aroma. The Mann-Whitney further test showed that five different formula pairs and one pair had no difference. F1 (1.46) has a very low burned aroma compared to F2 (1.71), F3 (2.26), and F4 (2.36), which has a slightly lower burned aroma. The burning smell arises because the fish protein hydrolyzate undergoes a hydrolysis process at high temperatures and a long time to break down the protein into simpler peptides so that it can cause the Maillard reaction, namely the reaction between amino acids and carbohydrates [33].

The intensity of the fishy aroma of pudding ranged between 1.61-3.47. The statistical test results showed that there were six different formulation pairs, and the intensity of F1 (1.61) and F2 (2.33) had a slightly lower fishy aroma compared to F3 (3.12) and F4 (3.47), which had a moderate fishy aroma. The resulting fishy aroma can affect the intensity of the fruit and milk taste of the pudding due to the dominant fishy aroma of FPH. The fishy aroma that is produced occurs during the fat hydrolysis process [34], which can damage the fat and produce a fishy fish aroma or an unpleasant aroma which is caused by decomposition where the sulfhydryl group is easily separated from the protein and forms H_2S which can form volatile compounds or fishy odors.

The intensity of the aroma of milk aroma statistics show that five pairs of formulations are different, and one other pair has no significant difference. Based on the intensity values, F1 (3.44), F2 (3.00), and F3 (2.70) are moderate, and F4 (2.51) is relatively low. The aroma of milk occurs when the milk fermentation process uses lactic acid bacteria. Compounds that form milk aroma include lactose, cystine, fatty acids, and folate compounds. Volatile aroma compounds, namely methionine and aromatic amino acids, produce other compounds that can cause aroma [35]. The bitter taste intensity ranges from 1.66-2.17, with the Mann-Whitney Test indicating differences in 2 pairs of formulations and no significant differences in 4 pairs. Notably, F1 (1.66), F2 (1.90), F3 (1.99), and F4 (2.17) produce a low bitter taste. The enzymatically hydrolyzed FPH is a key contributor to the dominant bitter taste, which is a characteristic of this type of protein. The bitter taste also arises during the process of separating fat and protein, and FPH produces dissolved protein, free amino acids, and methionine, valine, isoleucine, and leucine [36].

The bitter taste of peptides is due to the hydrophobicity of the phenylalanine or tyrosine molecules. The volatile compound 2-piperidine can also contribute to the bitter taste, but phenylalanine and tryptophan produce a higher bitter taste [37]. The intensity of the salty taste ranges from 1.42-1.70. The addition of FPH had no effect, as shown by the results of the Kruskal-Wallis statistical test. Based on the salty taste intensity value of the pudding in formulations F1 (1.42) and F2 (1.54) give rise to a low salty taste, while F3 (1.63) and F4 (1.70) give a slightly low salty taste. The salty taste of pudding experiences the formation of oligopeptides [38].

FPH has the same amount of amino acid protein as monosodium glutamate (MSG), so its addition can cause a salty or umami taste, even if only slightly [39]. The Maillard reaction can produce a salty or sour taste [40]. The addition of hydrolyzate affected the sweet taste, as indicated by the results of the Mann-Whitney test, which showed five different formulation pairs and one pair that had no difference. Based on the sweetness intensity value, pudding with formulations F1 (3.46), F2 (3.17), F3 (2.88), and F4 (2.74) produces a moderately sweet taste. The sweet taste tends not to come from FPH and sugar. Compounds that form a sweet taste from FPH include V (valine), G (glycine), P (proline), A (alanine), and K (lysine) [41].The results of the Mann-Whitney statistical test, fruit flavor intensity, showed that four formulations differed, and two pairs had no differences.

Based on the fruit flavor intensity value, pudding with formulation F1 (3.70) produces a slightly high fruit taste, while F2 (3.17), F3 (2.94), and F4 (2.74) make a medium fruit taste. The fruity taste comes from the mango flour added to the pudding. The makeup manga flavor compounds include esters, terpenoid aldehydes, alcohols, and reducing acid sugars [42]. The statistical tests on color intensity revealed no significant differences among the formulations. The color intensity values for formulations F1 (3.50), F2 (3.46), F3 (3.40), and F4 (3.33) all fell within the medium range. The orange color of the pudding is a result of the addition of natural dyes and FPH, which contain crocin and crocetin compounds that produce the desired color, making the pudding similar to commercial ones [43]. The addition of FPH did not affect the elasticity of the pudding, as shown by the results of the Kruskal-Wallis statistical test. Based on the intensity value, formulations F1 (3.27), F2 (3.09), F3 (3.08), and F4 (3.02) have a medium chewy texture.

3.4 Hedonic quality testing

The hedonic quality test states the good or bad impression of a product based on the panelists' assessment of several typical parameters of a product such as color, aroma, taste and texture. The results of hedonic quality can be seen in Table 3.

Table 3 Hedonic quality testing

Attribute	F1	F2	F3	F4
Color	3.98±0.24	4.08±0.26	3.90±0.21	3.73±0.07
Aroma	$4.43±0.15^{c}$	$3.93±0.09^{b}$	$3.23±0.07^{a}$	$3.06±0.16^{a}$
Taste	$4.18±0.11^{c}$	$3.59±0.11^{b}$	$3.30±0.10^{ab}$	$3.09±0.08^{a}$
Texture	$4.28±0.17^{c}$	$3.92±0.05^{b}$	$3.87±0.03^{ab}$	$3.63±0.03^{a}$

Values with different superscript letters in the column indicate significant differences between treatments ($p<0.05$).

The hedonic color attribute ranges between 3.73 and 4.08, with the results of the Kruskal-Wallis statistical test having a not significance value of $p = 0.070$. The hedonic quality values of formulations F1 to F4 show that the pudding has an orange color and does not change with the addition of FPH. Consumers like food products that contain additives such as colorings. Consumers also need information about nutrition, diet, food composition, and food additives' functions, benefits, and safety [44]. The hedonic aroma attribute of the pudding, ranging between 3.06-4.43, is a result of the statistical test showing 6 different formula pairs and 1 formulation with no difference. The hedonic quality values of formulations F1 (4.43) and F2 (3.93) give rise to a typical aroma of mango and pudding, with a slight fishy smell, while F3 (3.23) and F4 (3.06) give rise to a stronger fishy smell. This aroma is a direct result of the ingredients used, which produce volatile compounds that evaporate in the product [45].

The hedonic taste attribute of the pudding, ranging between 3.09-4.18, is a result of the Mann-Whitney statistical test showing 5 different pairs, and 1 pair with no difference. The hedonic quality values of formulations F1 (4.18) and F2 (3.59) still give rise to a sweet, mango and milk taste, while F3 (3.30) and F4 (3.09) give a slightly sweet and bitter taste. The taste of the pudding decreases as the FPH concentration increases due to the dominant amino acids phenylalanine and threonine, which cause a bitter taste in FPH [13]. The hedonic of texture attribute looks soft texture of the pudding with statistical test results shows that there are 4 different pairs, and two pairs have no differences. Hedonic values F1 (4.28), F2 (3.92), F3 (3.87), and F4 (3.63) indicate a soft and slightly chewy texture. The texture produced from agar flour gel, which is added with a low value along with the FPH concentration added, is due to the colloids contained in FPH, which produce a slightly denser and harder texture [4].

3.5 Overall Hedonic testing

A hedonic test is a personal response from panelists about liking or disliking the product presented as a whole (overall). Hedonic results can be seen in Table 4.

Table 4 Hedonic overall testing

Formulation Code	**Overall Hedonic Testing**
F1	3.78 ± 0.15^{c}
F2	3.39 ± 0.17^{b}
F3	3.06 ± 0.17^{a}
F4	2.74 ± 0.05^{a}

Statistical tests show that all treatments are different. Panelists tend to prefer F1 pudding (control). This occurs in the fishy taste and aroma, and the addition of FPH causes the bitter taste of fish in the pudding. However, the panelists' preference for F2 (5%) is similar to the control (F1). Consumers typically make food choices based on enjoyment to meet their body's nutritional needs, so, food producers need to consider food that is nutritious and delicious [46].

4. Conclusion

In conclusion, our research demonstrates that the addition of higher FPH can indeed increase the protein content of the pudding. The highest protein content is achieved with the addition of 15% FPH. However, the pudding that is most preferred by panelists is the one with 5% FPH. This consumer acceptance factor serves as a crucial limitation on the choice of FPH concentration added to the pudding.

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