

Physicochemical Properties of Rice Fortified with Payangka Fish Protein Hydrolysate

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ABSTRACT

Rice is an energy source that is high in carbohydrates and contributes to providing the largest percentage of calories, but rice has a low protein, vitamin and mineral content, so it needs to be fortified to increase the nutritional content of rice, especially protein. This research aims to characterize rice that has been fortified with protein hydrolyzate. Synthesis of Payangka fish protein hydrolyzate was carried out using papain enzyme with concentrations of 2,4 and 6% with an incubation time of 2 hours. The best fortification of rice is done by soaking the rice for 4 hours, then drying it again for 14 hours at a temperature of 50°C. The research results show that Payangka fish hydrolyzate can be obtained through hydrolysis of Payangka fish meat using the Papain enzyme with a concentration of 2-6%. The highest protein content of protein hydrolyzate was obtained when using a Papain enzyme concentration of 6% with a protein content of 3.61%. Fortification of rice with Payangka fish protein hydrolyzate can increase rice protein content, namely from 9.43% (before fortification) to 10.38% (after fortification using protein hydrolyzate with a concentration of 30%)

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Introduction

Payangka fish is an endemic fish to Lake Tondano. Minahasa, North Sulawesi. Until now, payangka fish has not provided good economic value. The people of Minahasa and Manado in general also don't like the Payangka fish type. Payangka fish reproduction is very fast. Payangka production reaches around 35% of all fish production and dominates the catch in Lake Tondano, but unfortunately its economic value is low. Therefore, diversification of processed payangka fish products is expected to increase the economic value of payangka fish [1]

Fish protein hydrolyzate (HPI) is an engineered product of food processing technology carried out through a hydrolysis reaction which is able to convert fish into food products with high nutritional value. Protein hydrolyzate can be used as a source of food protein which can be widely applied to various foods to increase nutritional

content and prevent "stunting" has applied protein hydrolyzate to nugget to prevent stunting.

Stunting is a disruption in the growth and development of children due to chronic malnutrition and recurrent infections, which is characterized by their length or height being below the standards set by the minister who handles government affairs in the health sector. The accelerated implementation of stunting reduction includes target groups: teenagers, prospective brides, pregnant women, breastfeeding mothers, and children aged 0 - 59 months. Handling of stunting reduction has been regulated in Presidential Regulation (PERPRES) No.72 of 2021 concerning the Acceleration of Stunting Reduction [2].

According to the World Health Organization (2023) [3] the number of stunted children under five in the world in 2020 will be 22% or around 149.2 million children under five. Meanwhile,

Indonesia ranks 4th in the world and 2nd in Southeast Asia regarding cases of toddlers experiencing stunting. Based on SSGBI (Indonesian Toddler Nutrition Status Survey) data, in 2021 the prevalence of stunting in Indonesia is 24.4% or around 5.33 million children under five. One solution to overcome stunting children is by consuming fortified foods.

Based on the description above, the development of rice fortification in Indonesia has enormous potential and strategic value. Payangka fish protein hydrolyzate has great potential as a fortifier in rice because apart from being a staple food for Indonesian people, it is also supported by its abundant availability and its economic value is still very low.

Materials and Methods

The tools used in this research are meat bone separator, spinner, UV-Vis spectrophotometer, centrifuge, empty cup, oven, digital scales, sokhlet, Kjeldahl, Erlenmeyer.

The ingredients used in this research were rice of 4 different types from Kotamobagu city, Payangka fish, distilled water, Bromelin enzyme, Papain, Trichloroacetate (TCA), HCL, Potassium Borate, Glycerol Monostearate, Methanol, Sulfuric Acid, Borac Acid, Methylene Red, Methylene Blue, Sodium hydroxide, ophthaldialdehyde (OPA).

The research procedure is as follows:

1. Preparation of protein hydrolyzate

Aged Payangka fish is ground using a meat bone separator then put into a hydrolysis tank which has been filled with water with a ratio of (1:4) (w/v) papain with various concentrations (2, 4, and 6%), then hydrolysis is carried out with a temperature controlled between 55-60°C and pH 7. The hydrolysis process is carried out for 7 hours. Every hour of hydrolysis measure the degree of hydrolysis (DH). Next, the enzyme was inactivated at 90°C for 20 minutes, filtered, centrifuged at 4°C. The protein hydrolyzate formed was allowed to stand until two separate fractions were formed. The filtrate fraction and the resulting residue are separated by filtration using a spinner until a clear colored filtrate is obtained and then a proximate test is carried out.

2. Rice selection

The rice taken in the Kotamobagu area is 1 kg each with 4 different varieties. Each rice was

washed clean and then subjected to organoleptic and proximate tests

3. Rice fortification

In this research, fortification of rice with protein hydrolyzate was carried out using the Parboiling Technique [4] namely soaking the rice at a temperature of 70 °C for 2 hours with protein hydrolyzate with concentrations of 10, 30 and 50%, then the rice was re-ovened for 14 hours at a temperature of 50oC. Fortified rice is tested using the proximate test

4. Proximate test

- Water content analysis (SNI 01-2891-1992 item 5.2)
- Ash Content (SNI 01-2891-1992 item 6.1)
- Fat Content (SNI 01-2891-1992 item 8.2)
- Protein content (SNI ISO 1871-2015)
- Carbohydrate Content (IK-1.18 Carbohydrate calculation)

Results and Discussion

1. Payangka Fish Protein Hydrolyzate

In this research, Payangka fish protein hydrolyzate was synthesized using the Papain enzyme with varying concentrations, namely 2.4.6%. The resulting Payangka fish protein hydrolyzate is a yellowish liquid (Figure 1)



Figure 1. Payangka Fish Protein Hydrolyzate

The stages of protein hydrolyte synthesis are: incubation, inactivation and filtering. The incubation time used in this study was 2 hours. The researchers used different incubation times in the synthesis of protein hydrolysates. [5] synthesized soy protein hydrolyzate with varying incubation times, namely 0 hours; 1.5 hours; and 3 hours).

Fish Protein Hydrolyzate (HPI) is a product resulting from the breakdown of fish protein into short chain compounds due to a hydrolysis process

by enzymes, acids and bases. The raw material for fish protein hydrolyzate usually comes from unused fish waste and has a protein content that is still maintained [6]. Fish protein hydrolyzate can be produced chemically and enzymatically. Enzymatic hydrolysis is more efficient, cheaper, produces fish protein hydrolyzate without losing essential amino acids, and avoids product changes or damage.

2. Yield

The resulting HPI yield value varies depending on several things, such as the type of fish and processing techniques. Yield is one of the important parameters in the fishery product processing process which aims to estimate the number of parts of the raw material that can be utilized. According to [7] the percentage of the amount of hydrolyzate product produced to the volume of raw material before hydrolysis is called the yield of the hydrolyzate product. The yield of protein hydrolyzate is presented in table 1.

The yield of Payangka fish produced in this study was 95.4-95.7% (Table 1). The resulting yield value is different from the results of other studies. [8] using 3 fish species, obtained a yield for tilapia of 5.64%, milkfish 2.73%, shark 2.83%. The low yield of protein hydrolyzate obtained in this study was due to the procedure for making protein hydrolyzate, the process of concentrating/reducing water content was carried out using a spray drying technique [9]. Likewise, according to research results from Widadi (2011), the HPI yield of African catfish was 21.16%.

In this research, the Payangka fish protein hydrolyzate product was not concentrated because the Payangka fish protein hydrolyzate will be used for rice fortification.

Table 1. Yield of Payangka fish protein hydrolysate

Enzyme concentration(%)	Average yield (%)
2	95,4
4	95,5
6	95,7

3. Degree of Hydrolysis of Payangka fish

The degree of hydrolysis of Payangka fish protein is presented in table 2. Based on table 2 it is known that increase in the total amount of nitrogen in the the protein hydrolyzate ranges from 95.71-97.24%. This shows that the degree of hydrolysis of Payangka enzyme concentration increases, the speed of the fish increases with increasing Papain enzyme hydrolysis reaction increases, however, to a certain

concentration. This indicates that the hydrolysis process is maximizing

Table 2. Degree of hydrolysis of Payangka fish

Enzyme concentration(%)	Degree of Hydrolysis
2	96,21
4	96,99
6	97,51

The DH value is calculated using the formula:

$$DH = \frac{\text{dissolved Absorbent TCA 6,25\%}}{\text{Undissolved Absorbent TCA 6,25\%}} \times 100\%$$

The degree of protein hydrolysis (DH) is determined by several factors including the type of protease used, enzyme concentration, temperature, pH and hydrolysis time [10,11] Therefore, it is very important to optimize several of these factors to obtain optimal DH. (restiani 2016).

4. Payangka Fish Hydrolyzate Proximate Test Results

Table 3. Proximate test results of Payangka fish protein hydrolysate

Parameter	Unit	Papain enzyme concentration		
		2%	4%	6%
Total Protein	%	3,33	3,45	3,61
Fat	%	0,52	0,47	0,57
Water Content	%	90,67	91,45	87,90
Ash Content	%	0,02	0,003	0,04
Coarse Fiber	%	0,05	0,09	0,08
Karbohidrat	%	5,46	4,60	7,88

a. Total protein content

The results of protein content analysis showed that the level of Payangka fish hydrolyzate made with a 2% Papain enzyme concentration was 3.33%, and the protein content of Payangka fish hydrolyzate made with 4% Papain concentration was 3.45, while the protein content of Payangka fish hydrolyzate made with 6% Papain concentration was 3.61%. This shows that adding the Papain enzyme concentration to the hydrolysis of Payangka fish meat has an effect on the levels of fish protein hydrolyzate. Protein content increased as the concentration of added enzyme increased.

The increase in protein value indicates an analysis method used. This shows that as the This shows that the degree of hydrolysis of Payangka enzyme concentration increases, the speed of the fish increases with increasing Papain enzyme hydrolysis reaction increases, however, to a certain

extent, excessive addition of enzyme can result in a constant amount of hydrolyzate because the added enzyme is no longer active. Excessive use of enzymes does not have an effect on the value of the dissolved protein because during the process there is no addition of substrate and the available substrate has been used up during the hydrolysis process [12]. The increase in protein content is thought to be caused by the detection of enzymes in the treatment. Enzymes are proteins and their catalytic activity depends on the integrity of their structure as proteins [13]

b. Fat Content

The fat content obtained at a 2% concentration was 0.52%, a 4% concentration was 0.47%, and the highest level was obtained at a 6% concentration of 0.57%. The fat content of Payangka fish is quite low, below 1%.

Fat is an important element in food which functions as an energy source. The fat content should be low so that oxidation does not occur easily so that it has a long shelf life. The fat content in protein hydrolyzate is quite an important component. Fish protein hydrolyzate products with low fat content generally have a more stable and long-lasting quality compared to hydrolyzate products with high fat content. Most fish protein hydrolysates have a fat content of less than 5% [14,15]

c. Water content

The principle of water content analysis is the process of evaporating water from a material by heating. Determination of water content is based on the difference in sample weight before and after drying. The water content of the protein hydrolyzate made with a Papain enzyme concentration of 4% has the highest water content value, namely 91.45%, while the lowest level is the protein hydrolyzate made with a concentration of 6% with a water content of 87.90%. The water content of the fish protein hydrolyzate according [14] ranges from 1-8%. The water content of fish protein hydrolyzate is below 10% [15]

affected by the drying method of fish protein hydrolyzate. Protein hydrolyzate drying methods usually use Spray Drey [16] and Freeze Drying [17].

The high water content of Payangka fish protein hydrolyzate in this research is because drying was not carried out in this research as in

other studies because the resulting protein hydrolyzate will be used to fortify rice by soaking.

In [18] and [19] showed that the HPI water content value of African catfish was 5.46% using the spray drying method and the water content value of white snapper innards hydrolyzate was 10.82%. According to [20] the higher the drying air temperature, the greater the heat carried by the air so that more water vapor is evaporated from the surface of the material being dried. Low water content will cause the powder to become more hygroscopic so that there is a large difference in water vapor pressure between the solid and the liquid.

d. Ash Content

The ash content of the Payangka fish protein hydrolyzate made with an enzyme concentration of 2% was 0.02%, while at an enzyme concentration of 4% an ash content was obtained at 0.03% and at a Papain enzyme concentration of 6% an ash content was obtained at 0.04%.

In several other studies, the highest ash content in *Thunnus thynnus* fish protein hydrolyzate was 13.7%. High ash content indicates high inorganic residue content and low organic matter recovery [21]. In addition, [22] reported that the high ash content in fish protein hydrolyzate was caused by the addition of alkaline compounds such as NaOH and acidic compounds such as HCl. According to [23] the high ash content was caused by the addition of NaOH in the hydrolysis process to adjust the pH and remaining fish bones. In addition, the mixture of acidic and basic compounds in the protein hydrolyzate solvent causes the formation of salt compounds which increase the ash content of the protein hydrolyzate [24]

The ash content of fish protein hydrolyzate according to [14] is 1-8%. Ash content according to [15] states that the hydrolyzate contains ash content of 0.45% -27% of the total amount of proximate. From the total amount obtained from 3 different Payangka fish protein hydrolyzate ash content concentrations. In this study, the part of the fish used was fish meat, while the high ash content was mostly obtained from the bones in each part of the fish waste including the head, fins, tail [25]

e. Crude Fiber

The rough density of Payangka fish protein hydrolyzate made with an enzyme concentration

of 4% is 0.09%, which is higher than the enzyme concentrations of 2% and 6%. The fiber content in Fish Protein Hydrolyzate (HPI) is generally low compared to fiber in other food ingredients.

f. Carbohydrate

The carbohydrate content of Payangka fish hydrolyzate made with the Papain enzyme at a concentration of 2% was 5.46%, the 4% enzyme concentration was 4.60% and the 6% concentration was 7.88%. Carbohydrate levels at a concentration of 6% were compared to higher than in several previous studies obtained a carbohydrate content of Milkfish protein hydrolyzate of 2.35%, much lower than the research results of [26] which reported that the carbohydrate value of Catla fish reached 4.88%. Meanwhile, [9] added that the carbohydrate content of hydrolyzed white snapper innards reached 20.39%, which indicates the presence of glycogen

5. Determination of the best rice

Table 4. Rice proximate test result

Parameter	Unit	Serayu	Pandan	Apel	Superwin
water content	%	11,22	12,67	11,11	11,82
Ash Content	%	0,60	0,64	0,50	0,5
Protein	%	9,43	9,15	7,70	8,58
Fat content	%	1,98	1,43	1,21	1,38
cruide fiber	%	0,28	0,27	0,37	0,34
carbohhydrate	%	76,77	76,11	79,66	77,66

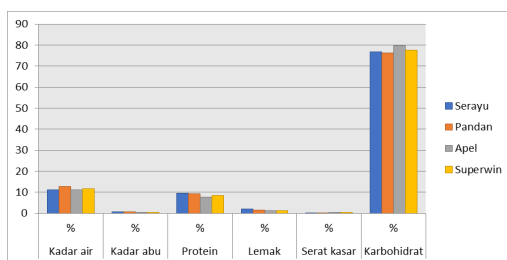


Figure 2. Bar diagram of proximate test results for each rice

In the table and diagram above, the best rice was obtained by carrying out a proximate test, Serayu rice was obtained with a protein content of 9.43%. The protein content of Serayu rice is higher than the protein content of the three other types of rice. When compared to several previous studies that used other types of rice, such as [27] used analog rice with a protein content of 1.88%, and research by [28] used analog rice with a protein

content of 5.45%. Based on this, Serayu rice will be fortified with payangka fish protein hydrolyzate.

Scientific research regarding the Serayu type of rice is not widely known because this rice is still one of the local products of the Bolaangmongondow community, especially in the city of Kotamobagu.

6. Rice fortification

In this research, rice fortification was carried out using the technique[4]. As much as 1000g of rice flour was added with protein hydrolyzate with varying concentrations, namely 10%, 30% and 50%.

The fortifier used in rice fortification must be able to be absorbed by the body, stable during the storage process and the rice cooking process. One form of improving intrinsic micronutrients in rice is the introduction of the concept of parboiling [29]. Initially the rice grains are soaked, steamed for a few minutes, and dried again. During this process, the nutritional content in rice such as vitamins B1, B6, and niacin in the endosperm will increase threefold. This is because the vitamin migrates from the bran into the endosperm [29]. Fortified rice is dried to a moisture content of 14% to increase stability during storage. The fortification rice coating process can increase the retention and stability of rice so that it can reduce micronutrient deficiencies during the rice washing process [30].

7. Rice test results after fortification Proximate test results

Table 5. Proximate test results for rice after fortification with payangka fish protein hydrolyzate

Parameter	Unit	Serayu HP 0%	Pandan HP 10%	Apel HP 30%	Superwin HP 50%
water content	%	11,22	16,74	16,04	14,33
Ash Content	%	0,60	0,57	0,48	0,58
Protein	%	9,43	9,70	10,38	10,36
Fat content	%	1,98	0,80	1,15	1,19
cruide fiber	%	0,28	0,29	0,30	0,33
carbohhydrate	%	76,77	72,19	71,95	73,53

Based on table 5, it is known that rice that has been fortified with Payangka fish protein hydrolyzate changes its nutritional content. The water content of fortified rice is higher than unfortified rice, which shows that the rice drying process is still not optimal. Water content greatly influences other nutritional levels, such as protein,

fat, ash and carbohydrate levels

The highest protein content of fortified rice was found in rice fortified with 30% protein hydrolyzate with a protein content of 10.38%. The protein content in rice is much higher than the protein hydrolyzate content in fish and rice before fortification. This shows that the addition of Payangka fish protein hydrolyzate has an influence on protein levels and increases the nutritional value of rice, especially Serayu rice.

Fish protein hydrolyzate is a product resulting from the breakdown of fish protein into short chain compounds due to the hydrolysis process by enzymes, acids and bases [6]. Protein hydrolyzate is generally used as an alternative protein source in commercial products [31]. The high protein content in fish protein hydrolyzate has the potential to be used as a supplement to meet human nutrition [15]. Fish protein hydrolyzate can be used as a fortification ingredient for various foods [5].

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Conclusion

Payangka fish hydrolyzate can be obtained through hydrolysis of Payangka fish meat using the Papain enzyme at a concentration of 2-6%. The highest protein content of protein hydrolyzate was obtained when using a Papain enzyme concentration of 6% with a protein content of 3.61%

Fortification of rice with Payangka fish protein hydrolyzate can increase rice protein content, namely from 9.43% (before fortification) to 10.38% (after fortification using protein hydrolyzate with a concentration of 30%).

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