International Journal of Research in Engineering and Modern Technology (IJREMTE)
Volume 1, No 1, October 2024, Pages 9 - 18
ISSN 3089-4077

Impact of Low Temperature Storage and Frozen *Litopenaeus vannamei* Head Protein Hydrolysate Concentration on the Quality of Rice Bran Flour

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Protein hydrolysates are proteins that undergo hydrolytic degradation with acids, bases, or proteolytic enzymes, resulting in amino acids and peptides. Protein hydrolysates have several uses in the food and pharmaceutical industries. Research shows that fish protein hydrolysates are used as food additives in soups, broths, sausage seasonings, biscuits, and crackers. Additionally, protein hydrolysates can be included in the diets of patients with digestive disorders. Rice bran is a byproduct of rice milling derived from various rice varieties. It is the outer part of the grain, including a small portion of starchy endosperm. The acquisition of rice bran involves several stages in the rice milling process, initially obtained from brown rice with byproducts including husks and coarse bran. The functionality of rice bran flour is similar to that of wheat flour, with functional properties such as water solubility, water absorption, fat absorption, and others. The aim of this study is to determine the effect of adding rice bran flour at different volumes of frozen Litopenaeus vannamei head protein hydrolysate. The method used in this research is an experimental method using a Randomized Block Design (RBD) with 3 replicates. Litopenaeus vannamei head protein hydrolysate, in volumes of 100 mL, 200 mL, and 300 mL, was added to 200 g of rice bran and stored at room temperature. Proximate analysis was performed on days 0, 5, 10, and 15. The analyses included protein content, fat content, water content, ash content, carbohydrate content, pH, and yield. The results of the study showed that the addition of rice bran at different volumes of Litopenaeus vannamei head protein hydrolysate stored at low temperatures affected the parameters of protein content, fat content, water content, carbohydrate content, pH, and yield, while the ash content parameter was not affected.

Keywords— Protein Hydrolysates, Fish Protein Hydrolysates, Rice Bran, *Litopenaeus vannamei*

I. Introduction

Protein hydrolysates are proteins that have undergone hydrolytic degradation with acids, bases, or proteolytic enzymes, resulting in amino acids and peptides. Protein hydrolysates have various uses in the food and pharmaceutical industries. Research shows that fish protein hydrolysates are used as food additives in soups, broths, sausage seasonings, biscuits, and crackers. Additionally, protein hydrolysates can be included in diets for patients with digestive disorders Protein hydrolysates can be in liquid, paste, or powder form. According to [2], liquid protein hydrolysates are prone to spoilage due to their high water content, which also affects their short shelf life and makes them difficult to transport. One way to address this issue is by adding rice bran to fish protein hydrolysates, thus converting them into flour or paste.

Rice bran is a byproduct of rice milling derived from various rice varieties. It is the outer part of the grain, including a small portion of starchy endosperm [3]. The acquisition of rice bran involves several stages in the rice milling process, initially obtained from brown rice with byproducts including husks and coarse bran [4]. However, because rice milling equipment does not separate coarse bran from rice bran, they are generally mixed together and referred to as bran or rice bran [4]. The function of rice bran flour is still related to its functional properties, which are similar to those of wheat flour. These functional properties include the ability of rice bran to dissolve in water, absorb water, absorb fat, and so on [5]

The addition of rice bran in emulsion products can increase emulsion stability because rice bran has water-absorbing properties. The fiber in rice bran has a high water absorption capacity, which affects the water absorption process and prevents maximal gelatinization [6]. Additionally, carbohydrates in rice bran flour play a role in water binding and texture formation [7].

The carbohydrates in rice bran include cellulose, hemicellulose, and starch. The starch content in rice bran comes from the endosperm part that is carried over during the milling process [8] According to Damayanthi [9] the starch content increases with the number of milling stages performed.

As normal starch, its use is limited in the pharmaceutical industry due to its non-supportive characteristics, such as poor flowability and lack of binding properties, so it is only used as a tablet filler for drugs with good flowability or as a musilago, a binding agent in the wet granulation tablet-making process [10].

So far, there has been no research on the effect of adding rice bran flour to the hydrolysate of vaname shrimp heads (*Litopenaeus vannamei*) during low-temperature storage. Therefore, research on this topic is needed. From the explanations provided, it is necessary to study the utilization of rice bran flour on the quality of vaname shrimp head hydrolysate during storage

II. Literature Review

A. Vannamei Shrimp (*Litopenaeus vannamei*)

Vannamei shrimp is a marine organism belonging to the order Decapoda, where the shrimp's body is divided into three parts: head, thorax, and abdomen [11]. Shrimp belong to the phylum Arthropoda, subphylum Mandibulata, and class Crustacea. Their entire body consists of segments encased in an exoskeleton made of chitin, reinforced with calcium carbonate [12]. According to [13], vannamei shrimp have a translucent white body, hence often referred to as white shrimp. The size of vannamei shrimp is relatively small, distinguishing them from other shrimp species. The classification of vannamei shrimp is as follows:

- Phylum: Arthropoda

- Class: Crustacea

- Order: Decapoda

- Family: Penaeidae

- Genus: Litopenaeus

- Species: *Litopenaeus vannamei*

Shrimp is a highly favored Indonesian fishery commodity worldwide. One popular shrimp product is frozen vannamei shrimp. Processed shrimp products from the shrimp freezing industry include head-on (whole shrimp), headless (shrimp without the head), and peeled (shrimp without the head and shell). Potential industrial waste from headless and peeled shrimp products consists of the head and shell. And shrimp skin which consists of 30-50% of overall weight of the shirmp

B. Protein Hydrolysate

Fish protein hydrolysate is produced through the decomposition of fish protein into simple peptides or amino acids via hydrolysis using enzymes, acids, or bases. Fish protein hydrolysate can be used as a flavor enhancer, a source of protein, and amino acids in food ingredients. Fish protein hydrolysate of lower quality can be utilized as a protein source in animal feed, a nitrogen source in plant fertilizers, and as a growth medium for bacteria [14] .The uses of protein hydrolysate in the food industry include fortification in non-allergenic food formulations for infants, dietary supplements, and as an emulsifying agent [15]. According to [16], protein hydrolysates can be used to improve the characteristics of various food products and as flavor enhancers. In the pharmaceutical field, according to [1], they can be used in the production of dermatological products, such as facial cleansers and skin moisturizers. Protein hydrolysate technology was first introduced in China and Japan around 1990 and is a by-product in the production of Monosodium Glutamate (MSG). Protein hydrolysate is obtained after the crystallization process of MSG is completed, leaving neutralized and dried amino acids. Protein hydrolysate can be in liquid, paste, or powder form and is hygroscopic. Liquid protein hydrolysate contains 30% solids, while the paste form contains 65% solids [17]

The factors influencing the hydrolysis rate and the uniqueness of the product in the protein hydrolysate production process are temperature, hydrolysis time, and the concentration of the enzyme added. The extent of amino acid damage is affected by the purity of the initial protein material and the conditions and type of hydrolyzing agents used. The duration of the hydrolysis process is the most influential factor on the quality of the resulting hydrolysate [18].

Protein hydrolysate products have a bitter taste caused by short-chain peptides produced from protein breakdown. The sweet taste in protein hydrolysate is due to the amino acid glycine during hydrolysis, while the savory taste is caused by the formation of high levels of oligopeptides from glutamic acid during the hydrolysis process [19].

Fish protein hydrolysate plays a crucial role in improving the functional properties and quality of food ingredients. Fish protein hydrolysate has a high protein content, complete amino acids, high protein digestibility, and important functional properties in food processing, such as being a flavor enhancer, having high water solubility, and forming textures [20]. The color of protein hydrolysate is determined by the pigments/coloring agents present in the sample used as the raw material. The color of protein hydrolysate is also influenced by non-enzymatic browning reactions (Maillard reactions) during hydrolysis, which occur between the hydroxyl groups of sugars and the amino groups of amino acids or proteins [21].

The nutritional content of protein hydrolysate from Vannamei shrimp heads can be seen in Table 1 as follows:

Table.1 Nutritional content of *Litopanaeus vannamei* hydrolysate (Fathony, 2014)

Component	Content (%)
Protein	65,06 %
Water Content	16.06 %
Fat	2.59 %
Ash	12.94 %
Carbohydrate	3.37 %

C. Rice Bran Flour

Rice bran is a by-product of rice milling derived from various rice varieties. It comes from the outermost layer of the grain, including a small portion of starchy endosperm [22]. Rice bran is obtained through several stages in the rice milling process, initially obtained from brown rice along with by-products such as husks and coarse bran [23]. However, since rice milling equipment does not separate bran and rice bran, they are generally mixed together and referred to simply as bran or rice bran [24].

The relatively high fat content in rice bran makes it less durable, causing it to quickly develop an odor and become rancid. The free fatty acid content will increase by 1% every hour when stored at room temperature [25]. The rancidity reaction is caused by enzymatic lipase hydrolysis and oxidative rancidity. In rice bran, rancidity occurs due to lipase hydrolyzing fats into fatty acids and glycerol. Free fatty acids are oxidized by the enzyme lipoxygenase into peroxides, ketones, and aldehydes. High rancidity affects the sensory acceptance of rice bran as a food ingredient [25].

To obtain non-rancid rice bran and extend its shelf life, rice bran must be processed immediately and treated to inactivate the lipase enzyme that causes rancidity. One technique that can be used is converting the rice bran into flour and immediately oven-drying it at 100°C for 15 minutes[25].

Rice bran has the potential to be developed as a functional food. This potential correlates positively with rice as the primary consumption of the Indonesian population. The utilization of waste from rice milling can be further processed into functional food. The use of rice bran as food is currently limited because it is easily spoiled by hydrolytic and oxidative activities of lipase enzymes that are naturally present in rice bran oil or by microbes. Nevertheless, rice bran has potential as a functional food because it contains high nutritional value and bioactive components such as oryzanol, tocopherol, and ferulic acid.

The advantages of rice bran include its relatively high carbohydrate content, ranging from 51 to 55 g/100 g, and protein content of 11 to 13 g/100 g. Compared to rice, rice bran has a higher lysine amino acid content. The fat content in rice bran is between 10 to 20 g/100 g. Rice bran is also rich in B-complex vitamins and vitamin E, and it is an excellent source of dietary fiber. Besides aiding in digestion, the presence of dietary fiber also helps in lowering blood cholesterol levels. Rice bran contains significant amounts of fiber, with cellulose content ranging from 8.7 to 11.4% and hemicellulose content from 9.6 to 12.8% [26].

According to [27], rice bran is rich in vitamin B15 (pangamic acid). In general, rice bran contains protein, minerals, fats (including essential fatty acids), dietary fiber, antioxidants, vitamin E, and B-complex vitamins (such as B1, B2, B3, B5, B6, and B15). Additionally rice bran contains various bioactive compounds such as oryzanol, tocopherol, and ferulic acid, which contribute to its health benefits. These compounds have antioxidant properties, which help in protecting cells from oxidative damage and may reduce the risk of chronic diseases. The comprehensive nutritional profile and bioactive components make rice bran a valuable addition to the diet, promoting overall health and well-being.

III. Materials and Methods

A. Materials

The materials used are frozen shrimp head protein hydrolysate. The ingredients used for making the colloid system binder consist of rice bran flour, shrimp head protein hydrolysate, rubber, and cloth. The materials used for chemical analysis include silica gel, mattress thread, filter paper, H2SO4, Kjeldahl tablets, distilled water, NaOH, HCl, methyl orange indicator, PE (petroleum ether) solution, and label paper. The equipment used in the production of marine yeast culture consists of glass bottles, stove, boiling pot, spatula, volumetric pipette, suction ball, digital scale, aerator, funnel, measuring glass, and beaker glass. The tools used for counting the density of marine yeast cells consist of a microscope, Petroff-Hauser Chamber (Haemocytometer), micropipette, cover glass, reaction tube rack, reaction tube, suction ball, volumetric pipette, Erlenmeyer flask, measuring glass, digital scale, spatula, and sprayer. The equipment used for making rice bran protein hydrolysate consists of a basin, volumetric pipette, suction ball, measuring glass, and spoon. The tools used for chemical analysis consist of a pH meter, oven, desiccator, crucible tongs, goldfish, sample tube, cup glass, measuring glass, funnel, digital scale, dropper pipette, volumetric pipette, suction ball, Petri dish, reaction tube, reaction tube rack, beaker glass, porcelain cup, destruction, distillation, stand, burette, hot plate, and muffle.

B. Methods

The research method used in this study is the experimental method. Experimental research is intentionally conducted by researchers on variables for which data are not yet available, thus requiring manipulation through specific treatments to observe their effects [28]. The purpose of experimental research is to investigate the extent and causality by providing specific treatments to experimental groups and providing controls for comparison. This study used protein hydrolysate from Vannamei shrimp heads, which was already available from previous research, and added rice bran for proximate analysis.

Variables are factors that play a role or have an influence on an experiment. According to [28], variables are facts (data) whose values can vary or change. Variables are categorized into independent variables, dependent variables, and control variables. Independent variables are those that are manipulated to observe their effect on the dependent variables. Dependent variables are the outcomes that are typically the focus of the study. Control variables are variables that affect the dependent variable but are controlled or held constant. In this research, there are two variables: independent and dependent variables. The independent variables in this study are the volume of protein hydrolysate and storage duration. The volumes of protein hydrolysate used are 100 mL, 200 mL, and 300 mL,The dependent variables in this study are the proximate analysis of rice bran, including moisture content, fat content, ash content, protein content, and carbohydrate content. The results of all treatments will be stored at a low temperature.

The main procedure in this research involves mixing rice bran with Vannamei shrimp head protein hydrolysate. Samples of the Vannamei shrimp head protein hydrolysate are already available frozen and ready for use. The first step begins with weighing 200 grams of rice bran flour, which is then placed into a basin. Subsequently, 100 mL, 200 mL, and 300 mL of shrimp head protein hydrolysate are added. The purpose of adding hydrolysate with different concentrations is to determine its effectiveness in the process of producing rice bran protein hydrolysate. All treatments are repeated three times. All treatments are stored in a refrigerated room (low temperature) and observations are made on days 0, 5, 10, and 15, followed by proximate analysis (moisture content, fat, carbohydrates, protein). The schematic of mixing rice bran flour with Vannamei shrimp head hydrolysate can be seen in Figure 1

International Journal of Research in Engineering and Modern Technology (IJREMTE)
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ISSN 3089-4077

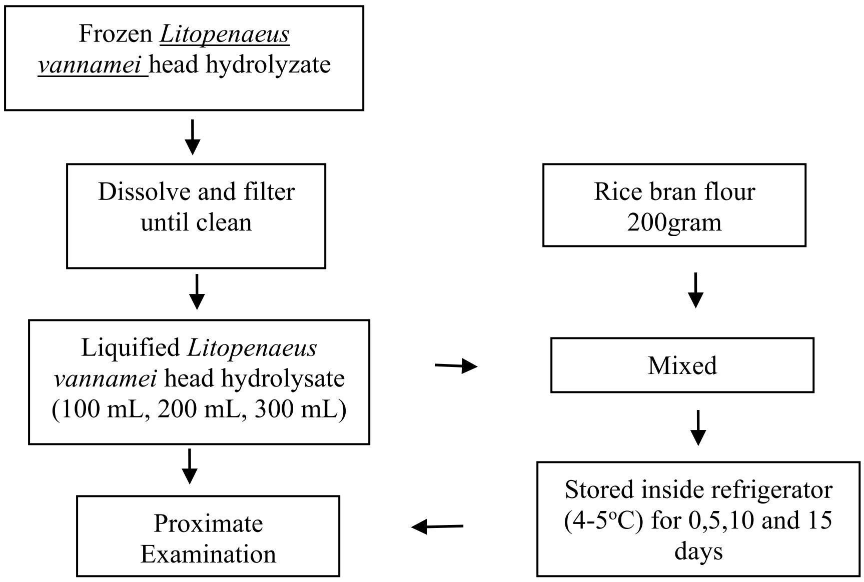

Figure 1 Research Project Flowchart

The experimental design used in this research is a Randomized Complete Block Design (RCBD) with 3 replications. The experimental design is as follows and can be seen in Tables 2

$$Y_{ij} = \mu + T_i + €_{ij}$$

Where:
Y_{ij} = response or observed value from treatment i and replication j
μ = overall mean value
T_i = effect of treatment i
$€_{ij}$ = experimental error effect from treatment i and replication j

Table 2 Randomized Complete Block Design (RCBD)

Treatment (Volume)													
Repeatation	I	II	III	I	II	III	I	II	III	I	II	III	
100 mL	1A	1B	1C	1A	1B	1C	1A	1B	1C	1A	1B	1C	
200 mL	2A	2B	2C	2A	2B	2C	2A	2B	2C	2A	2B	2C	
300mL	3A	3B	3C	3A	3B	3C	3A	3B	3C	3A	3B	3C	

To determine the effect of treatments on the measured response, analysis of variance (ANOVA) is conducted. If significant results are found, post hoc testing using Tukey's Honestly Significant Difference (HSD) test at the 5% significance level is performed using Microsoft Excel version 2013.

IV. Result and Discussion

A. Results

Proximate testing is an analysis of the content present in a substance, which includes moisture content, fat content, protein content, and carbohydrate content. Proximate testing is conducted to determine the nutritional composition present in shrimp head protein hydrolysate and rice bran flour. The results of proximate testing of shrimp head protein hydrolysate rice bran flour can be seen in Table 3 and 4 below.

Table 3 proximate testing results of shrimp head protein hydrolysate

Sample	Repetition	Fat Content	Protein	Water Content	Ash	Carbohydrate
Frozen *Litopenaeus vannamei* head hydrolysate	1	0.149	28.082	60.73	8.92	2.119
	2	0.148	30.039	59.057	8.873	1.883

Table 4 proximate testing results of rice bran flour

Sample	Fat Content	Protein	Water Content	Ash	Carbohydrate
Rice Bran Flour	10.64	13.82	11.44	9.82	55.86

B. Discussion

1) Fat Content Analysis

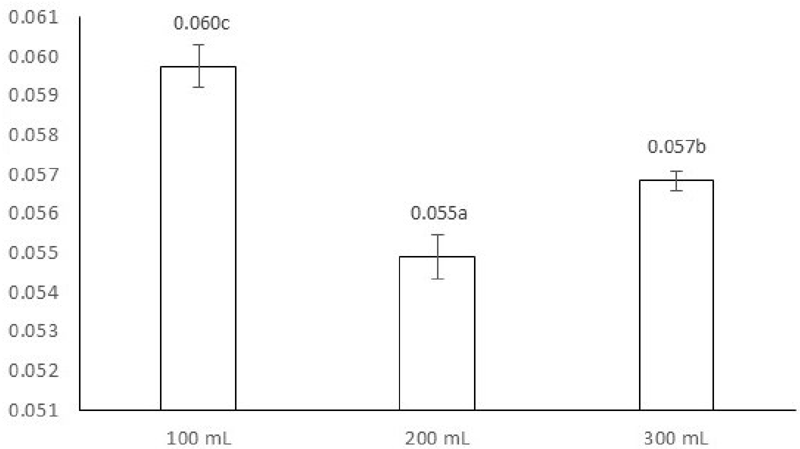

Figure 2 Fat Content of Shrimp Head Protein Hydrolysate with Various Volume Additions

Figure 2 shows that different volumes of *Litopenaeus vannamei* head protein hydrolysate result in different fat content. With 100 mL, the fat content was 5.98%; with 200 mL, it was 5.49%; and with 300 mL, it was 5.68%. Compared to the proximate test results of rice bran flour, the results showed a decrease, likely due to the fat hydrolysis process that breaks down fats into fatty acids and glycerol. This is supported by , who stated that fatty acids are obtained through the hydrolysis of natural fats because fatty acids are always found in nature bound to glycerol. Saturated fatty acids are those without double bonds, such as capric acid and lauric acid.

Compared to room temperature storage results reported by [29], the fat content of the shrimp head protein hydrolysate and rice bran mixture at different concentrations ranged from 9.28% to 8.44%. The highest fat content at a concentration of 300 mL was 9.28%, and the lowest at a concentration of 100 mL was 8.44%. The mixture of rice bran and shrimp head protein hydrolysate stored at cold temperatures had lower fat content, possibly due to the less optimal hydrolysis process that converts fats into fatty acids and glycerol. Additionally, the high moisture content during cold storage also had an impact. This is supported by [30], who stated that the lower the moisture content, the higher the fat content, and vice versa.

2) Protein Content Analysis

The observation data and analysis of the protein content of vannamei shrimp head protein hydrolysate mixed with rice bran flour, with varying volumes of hydrolysate. The data analysis results show that neither the addition of different volumes of hydrolysate nor the storage duration had an effect on the average ash content ($p<0.05$).

3) Water Content analysis

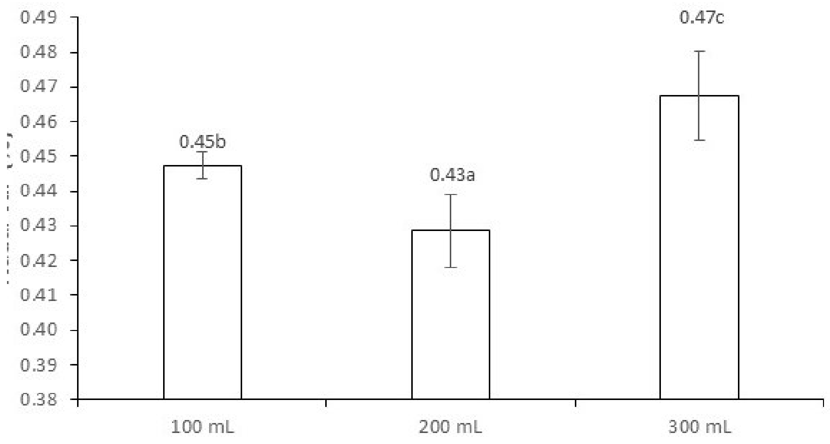

Figure 3 Water Content of Shrimp Head Protein Hydrolysate with Various Volume Additions

Figure 3 shows that the moisture content decreases compared to the proximate analysis of the moisture content of rice bran flour. This is likely due to the inherent properties of rice bran flour, which can absorb water well. According to [31], defatted extruded rice bran has better water absorption, oil absorption, foaming capacity, and foam stability compared to wheat bran. [32] also added that rice bran absorbed about 200 g of water per 100 g of bran in model tests.

Comparing the results with room temperature storage as reported by [29], the moisture content of the mixture of shrimp head protein hydrolysate and rice bran at different concentrations ranges from 37.40% to 35.41%. The highest moisture content at a concentration of 300 mL was 37.40%, and the lowest at a concentration of 100 mL was 35.41%. It can be seen that the moisture content in the mixture of rice bran and shrimp head protein hydrolysate is higher.

The high carbohydrate content in rice bran also plays a role in absorbing moisture. This is due to the carbohydrates themselves, such as hemicellulose, cellulose, and starch. This is supported by [33], who stated that carbohydrates in rice bran include cellulose, hemicellulose, and starch. The starch content in rice bran comes from the endosperm part that is carried over during the milling process [33]. Carbohydrates in rice bran flour play a role in binding water and forming texture [34].

4) Ash Content Analysis

The observation data and analysis of the ash content of vannamei shrimp head protein hydrolysate mixed with rice bran flour, with varying volumes of hydrolysate durations. The data analysis results show that neither the addition of different volumes of hydrolysate nor the storage duration had an effect on the average ash content ($p<0.05$).

5) Carbohydrate Content Analysis

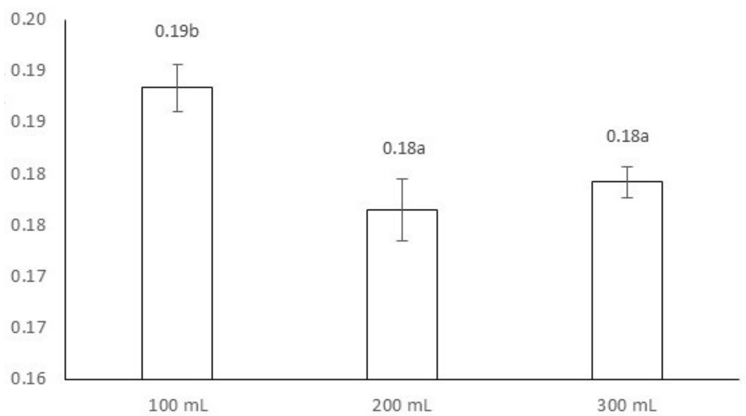

Figure 4 Carbohydrate Content of Shrimp Head Protein Hydrolysate with Various Volume Additions

Figure 4 shows that testing different concentrations of shrimp head protein hydrolysate resulted in different carbohydrate levels at each concentration. This is suspected to be due to the low carbohydrate content in shrimp head protein hydrolysate. Additionally, the increase in carbohydrates is thought to be because, according to [35], the reduction in fat due to microbial metabolism can increase the proportion of carbohydrates. This is further supported by the calculation of carbohydrate content based on the difference method. The increase in carbohydrate content is likely due to microbiological factors producing amylase enzymes, which can hydrolyze carbohydrates into simpler compounds such as polysaccharides, disaccharides, and monosaccharides, thus increasing the amount of carbohydrates. According to [36], ɑ-amylase enzymes can break down starch (amylum) into glucose. Amylase enzymes work by randomly hydrolyzing ɑ-1,4 bonds within the molecule, both in amylose and amylopectin. The initial hydrolysis by ɑ-amylase will produce dextrin, which is then further broken down into a mixture of glucose, maltose, maltotriose, and other longer chains.

Comparing this with room temperature storage, as reported by [29], the carbohydrate content of the mixture of shrimp head protein hydrolysate and rice bran at different concentrations ranges from 22.21% to 17.99%. The highest carbohydrate content at a concentration of 100 mL was 22.21%, and the lowest at a concentration of 300 mL was 17.99%. The mixture of rice bran and shrimp head protein hydrolysate stored at low temperatures resulted in lower carbohydrate content. This may be due to the minimal enzymatic reactions occurring at low temperatures, leading to lower results. Additionally, carbohydrate content is also influenced by other components. According to Sugito and Ari [36], the carbohydrate content is affected by other nutritional components; the higher the level of other nutritional components, the lower the carbohydrate content will be.

V. CONCLUSIONS

In the study titled "The Effect of Low-Temperature and Concentration of Frozen *Litopenaeus vannamei* Shrimp Head Protein Hydrolysate on the Quality of Rice Bran Flour," it can be concluded that the addition of *Litopenaeus vannamei* shrimp head protein hydrolysate to rice bran flour affects its quality. The addition of different volumes of hydrolysate during cold storage had a significant effect at a 5% error rate ($P>0.05$) on the average levels of protein, fat, water, carbohydrate, , but did not have a significant effect on the ash content parameter ($P<0.05$).

When compared to low-temperature storage, the overall results were lower. Therefore, for practical application, it is recommended to store the mixture at room temperature to achieve the desired improvements. Once the desired results are achieved, the mixture can be stored at low temperatures to maintain the quality of the obtained components.

CONFLICT OF INTEREST

The authors declare no conflict of interest".

AUTHOR CONTRIBUTIONS

Terry Previo Conduct Conceptualization and Research. Noven Indra, Lestari Ratnawati Provide Quantitative calculations and Statistics Results

REFERENCES

[1] Pigot and Tucker, "Fish Protein Hydrolysates as Food Additives," *Journal of Food Science*, vol. 55, no. 1, pp. 210-215, Mar. 1990.

[2] Shan et al., "Shelf Life of Liquid Protein Hydrolysates," *Food Chemistry*, vol. 141, no. 3, pp. 2305-2312, Nov. 2013.

[3] Hadipernata, "Rice Bran: Production and Utilization," *Rice Research Journal*, vol. 22, no. 4, pp. 145-150, Dec. 2007.

[4] Damardjati et al., "Rice Milling By-products and their Processing," *Journal of Agricultural Engineering*, vol. 32, no. 2, pp. 120-128, July 1989.

[5] Wulandari, "Functional Properties of Rice Bran," *Journal of Food Science and Technology*, vol. 40, no. 3, pp. 215-220, Sept. 2012.

[6] Damayanthi et al., "Water Absorption and Gelatinization of Rice Bran Flour," *Food Hydrocolloids*, vol. 21, no. 5, pp. 784-789, Aug. 2007.

[7] Liandani and Zubaidah, "Emulsion Stability in Food Products," *Journal of Food Engineering*, vol. 82, no. 4, pp. 418-424, May 2015.

[8] Parker, "Carbohydrates in Rice Bran," *Cereal Chemistry*, vol. 45, no. 2, pp. 176-182, Apr. 2003.

[9] Hargrove, "Starch Content in Rice Bran," *Journal of Agricultural and Food Chemistry*, vol. 42, no. 6, pp. 1302-1305, June 1994.

[10] Liberman et al., "Pharmaceutical Uses of Starch," *Drug Development and Industrial Pharmacy*, vol. 15, no. 7, pp. 987-995, Oct. 1989.

[11] Meiyani et al., "Biology and Utilization of Vannamei Shrimp," *Journal of Marine Biology*, vol. 30, no. 2, pp. 235-242, July 2014.

[12] Rosyidi et al., "Exoskeleton Composition of Vannamei Shrimp," *Crustacean Research*, vol. 18, no. 1, pp. 100-105, Jan. 2009.

[13] Amri and Kanna, "White Shrimp: Characteristics and Cultivation," *Aquaculture Journal*, vol. 14, no. 3, pp. 88-95, Sept. 2008.

[14] Kristinsson, "Fish Protein Hydrolysate: Production and Applications," *Journal of Food Biochemistry*, vol. 31, no. 5, pp. 656-671, Nov. 2007.

[15] Nurhayati, "Non-allergenic Food Formulations," *Journal of Nutrition and Dietetics*, vol. 18, no. 4, pp. 320-325, Dec. 2007.

[16] Pederson, "Improving Food Product Characteristics with Protein Hydrolysates," *Journal of Food Science and Technology*, vol. 12, no. 2, pp. 145-150, June 1994.

[17] Johnson and Peterson, "Liquid and Paste Forms of Protein Hydrolysate," *Journal of Agricultural and Food Chemistry*, vol. 22, no. 3, pp. 540-545, July 1974.

[18] Haslina, "Nutritional Value, Protein Digestibility, and Acceptance of Patilo as a Snack Food Enriched with Protein Hydrolysate from Mozambique Tilapia (Oreochromis mossambicus)," M.S. thesis, Diponegoro University, Semarang, Indonesia, 2004.

[19.] D. Budy, "Effect of Different Volumes of Boiled Molasses and Fermentation Time with Sea Yeast Starter on the Quality of Boiled Protein Hydrolysate from Vannamei Shrimp Heads (*Litopenaeus vannamei*)," B.S. thesis, Universitas Brawijaya, 2014.

[20] G. M. Hall and N. H. Ahmad, "Functional Properties of Fish Protein Hydrolysates," 1992.

[21] P. Bernadeta, P. Ardiningsih, and I. H. Silalahi, "Determination of Optimum Conditions for Protein Hydrolysate from Yellowtail Fish Waste (Caesio cuning) Based on Organoleptic Characteristics," J. Pengeluaran Kas, vol. 1, no. 1, pp. 26–30, 2012.

[22] M. Hadipernata, "Processing Rice Bran into Oil (Rice Bran Oil)," Warta Penelitian dan Pengembangan Pertanian, vol. 29, no. 4, pp. 8–10, 2007.

[23] D. S. Damardjati, B. A. S. Santosa, and S. Widowati, "Prospects for Developing Long-lasting Bran for Food Fortification," Risalah, 1987.

[24] K. Wulandari, R. Sulistijowati, and L. Mile, "Chitosan from Vannamei Shrimp Shells as Edible Coating for Tuna Fish Balls," Jurnal Ilmiah Perikanan dan Kelautan, vol. 3, no. 3, pp. 118–121, 2015.

[25] Janathan, "Physicochemical Characteristics of Rice Bran Flour and Optimization of Formulation and Shelf Life Prediction of a Mixed Beverage of Skim Milk and Rice Bran Flour," B.S. thesis, Faculty of Agricultural Technology, Bogor Agricultural University, Bogor, 2007.

[26] M. U. Azzmi, "Production of Red Rice Bran Noodle Substituted with Purple Sweet Potato Flour (Ipomoea batatas blackie) Rich in Antioxidants," Diploma III thesis, Faculty of Agriculture, Universitas Sebelas Maret, Surakarta, 2012.

[27] Y. Nursalim and Z. Y. Razali, Bekatul Makanan Yang Menyehatkan, Jakarta: PT. Agro Media Pustaka, 2007.

[28] A. Jaedun, "Experimental Research Methodology," Paper presented at In Service I Scientific Writing Training, Head of Puslit Dikdasmen, Faculty of Engineering, Universitas Negeri Yogyakarta, 2011.

[29] A. Munib, "Effect of Storage Duration and Different Concentrations of Hydrolysate on the Quality of Frozen Protein Hydrolysate from *Litopenaeus vannamei* Shrimp Heads," B.S. thesis, Faculty of Fisheries and Marine Science, Universitas Brawijaya, Malang, 2017.

[30] D. Purnanila, "Study of Preliminary Treatment on the Chemical Properties of Fish Meal Flour During Storage," B.S. thesis, Faculty of Agriculture, Universitas Sebelas Maret, Surakarta, 2010.

[31] C. James and S. Sloan, "Functional Properties of Edible Rice Bran in Model Systems," J. Food Sci., vol. 54, pp. 143–146, 1984.

[32] S. Barber and C. Benedito de Barber, "Rice Bran: Chemistry and Technology," in Rice: Production and Utilization, B. S. Luh, Ed., AVI Publishing Co., Westport, NY, pp. 791, 1980.

[33] K. L. Hargrove, "Processing and Utilization of Rice Bran in the United States," in Rice Science and Technology, W. E. Marshall and J. I. Wadsworth, Eds., Marcel Dekker, Inc., New York, 1994.

[34] R. Parker, Introduction to Food Science, Delmar, Thomson Learning, United States of America, pp. 15–21, 2003.

[35] T. Nurhayati, E. Salamah, and R. Nugraha, "Optimization of the Process for Producing Fish Offal Hydrolysate from White Snapper," PHP, vol. 17, no. 1, 2014.

[36] Sugito and H. Ari, "Addition of Gabus Fish (Ophicephallus Strianus) and Freezing Application in the Production of Gluten Pempek," Jurnal Ilmu - Ilmu Pertanian Indonesia, vol. 8, no. 2, pp. 147–151, 2006.