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THE EFFECT OF STORAGE DURATION OF SHRIMP HEAD PROTEIN HYDROLYSATE (*Litopenaeus vannamei*) WITH THE ADDITION OF RICE BRAN FLOUR AS A BINDER

Pengaruh Lama Penyimpanan Hidrolisat Protein Kepala Udang (*Litopenaeus vannamei)* Dengan Penambahan Tepung Bekatul Sebagai Bahan Pengikat

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ABSTRACT

This study aimed to evaluate the characteristics of shrimp head protein hydrolysate (*Litopenaeus vannamei*) with the addition of rice bran flour as a binding agent during specific storage periods. The research employed an experimental method with 5 variables and 5 replications, followed by statistical analysis using One-way ANOVA and Duncan's post-hoc test. The results showed that moisture and fat content tended to decrease over time, while ash and carbohydrate content increased. Protein content peaked on the 5th day before declining due to protein degradation. The findings suggest that the mixture of rice bran and shrimp head protein hydrolysate has potential as a raw material for protein supplements and carbohydrate-rich foods, depending on the storage duration.

Keywords: food supplement, protein hydrolysate, rice bran, shrimp head, storage

ABSTRAK

Penelitian ini bertujuan untuk mengevaluasi karakteristik hidrolisat protein kepala udang (*Litopenaeus vannamei*) dengan penambahan tepung bekatul sebagai bahan pengikat selama peri-ode penyimpanan tertentu. Metode penelitian menggunakan menggunakan metode experi-mental dengan 5 variabel dan 5 kali ulangan kemudian hasil diuji scara statistic dengan menggunakan One-way ANOVA dengan uji lanjut Duncan. Hasil menunjukkan bahwa kadar air dan lemak cenderung menurun seiring waktu, sedangkan kadar abu dan karbohidrat meningkat. Kadar protein mencapai puncaknya pada hari ke-5 sebelum mengalami penurunan akibat degradasi protein. Kesimpulan menunjukkan bahwa campuran tepung bekatul dan hi-drolisat protein kepala udang memiliki potensi sebagai bahan baku suplemen protein dan ma-kanan kaya karbohidrat, tergantung pada durasi penyimpanan.

Kata kunci: bekatul, hidrolisat protein, kepala udang, penyimpanan, suplemen makanan

INTRODUCTION

Whiteleg shrimp (*Litopenaeus vannamei*) is a high-value fishery commodity due to its rich nutritional content and diverse processing potential. Although the abdominal meat is widely used for human consumption, the head and tail are often discarded as processing waste that has not been optimally utilized.

Protein hydrolysates, produced through acid, base, or enzymatic hydrolysis of proteins, produce bioactive peptides and free amino acids with various applications in the food and pharmaceutical industries. Previous studies have documented the use of fish protein hydrolysates as functional ingredients in various food products such as soups, meat gravies, sausage seasonings, and bakery products (Pigot & Tucker, 1990). In addition, its high digestibility makes it very suitable for therapeutic diets for people with digestive disorders.

Rice bran, a by-product of rice milling derived from the outermost layer of rice grains (including the aleurone layer and part of the starchy endosperm), has physicochemical properties similar to wheat flour (Mulyana Hadipernata, 2007). Conventional rice milling processes usually produce a mixture of rice bran and rice bran (Damardjati et al., 1987; Wulandari, 2012) which shows good water and fat binding ability (Evy Damayanthi et al., 2007). When added to an emulsion system, rice bran increases stability through its good water absorption characteristics, although its high fiber content can interfere with the complete starch gelatinization process (Liandani & Zubaidah, 2015).

This study examines the functional properties of shrimp head protein hydrolysate combined with rice bran as a binder, with a particular emphasis on its potential application as a protein supplement or functional food additive. This study aims to increase the added value of shrimp processing waste through the development of innovative products while meeting nutritional needs in food formulations

RESEARCH METHODS

The materials used in this study were Shrimp head protein hydrolysate (*Litopenaeus vannamei*), Bran Flour, Mattress Thread, Filter paper, H_2SO_4, Aquades, NaOH, HCl, Petroleum ether, while the tools used in this study were pH meters, ovens, desiccators, baking sheets, crushable pliers, gold fisch, sample tubes, beakers, measuring cups, funnels, digital scales, droppers, volume pipettes, suction balls, petri dishes, spatulas, test tubes, test tube racks, beaker glasses, porcelain cups, destruction, distillation, stands, burettes, hot plates and muffles.

The method used in this study is to use proximate analysis consisting of analysis of water, ash, protein, fat, and carbohydrate content. Each analysis will be explained below:

a. Water Content Analysis Using Thermogravimetric Method (Sediaoetama, 2000)

A clean cup with an open lid is placed in an oven at a temperature of 105 C for 24 hours, then placed in a desiccator and allowed to cool for 15 minutes, the cooled cup is weighed empty, then weigh 15 grams of sample and placed in the cup, then put it in an oven at a temperature of 105 C for 3-5 hours, then cool in a desiccator for 15 minutes, weigh the cup again until constant then calculate using the formula.

$$\text{Water content}(\%) = \frac{(\text{weight of weighing bottle} + \text{sample}) - \text{final weight}}{\text{sample weight}} \times 100\%$$

b. Ash Content Analysis (Nuri Andarwulan et al., 2014)

Determination of ash content is done through a dry ashing technique using an electric furnace. This method works based on the principle of measuring the inorganic residue remaining after complete combustion of the organic components of the material at a

temperature of 550°C. The combustion process is carried out gradually without direct contact with the flame until a grayish-white ash with a constant weight is obtained.

Analysis Procedure:
1. Cup Preparation:
   - Porcelain cups are first conditioned in a furnace at a temperature of 105°C
   - Cooled in a desiccator for 15 minutes
   - Weighed to obtain the initial weight
2. Sample Preparation:
   - A sample of 5-10 grams is placed in a conditioned cup
   - Heated using a Bunsen burner with medium heat until:
   Smoke is no longer formed
   The color of the sample changes to black (perfect carbonization)
3. Ashing Process:
   - Sample is transferred to electric furnace
   - Heating is carried out gradually:
   * Phase I: 300°C for initial decomposition
   * Phase II: Increased gradually to 550°C
   * Total ashing time: 5-7 hours
4. Final Measurement:
   - The cup is carefully lifted using tongs
   - Cooled in a desiccator
   - Weighed until constant weight is obtained

c. Protein Content Analysis Using the Kjeldahl Method (Slamet Soedarmadji et al., 2007)

This study used the Kjeldahl method to determine protein levels, which consists of three main stages:
1. Destruction Stage:
   - The sample is heated in concentrated sulfuric acid (H2SO4) at high temperature
   - This process converts organic nitrogen into ammonium sulfate [(NH4)2SO4]
   - Organic compounds decompose into their constituent elements
2. Distillation Stage:
   - The destruction solution is made alkaline by adding NaOH
   - Ammonium sulfate is converted into ammonia (NH3) by heating
   - The ammonia vapor formed is distilled and captured in an absorbent solution
3. Titration Stage:
   - Using a standard acid solution (usually HCl)
   - Phenolphthalein (PP) indicator produces a color change to pink
   - The end point of titration is marked by:
   * Stable color change
   * Color lasts for at least 30 seconds
   - Protein content is calculated based on the amount of acid required

d. Analysis of Fat Content Using the Goldfisch Method (Slamet Soedarmadji et al., 2007)

Principle of Analysis
The Goldfish method is a continuous fat extraction technique using organic solvents. The working principle is based on the difference in solubility of lipid components in organic solvents at high temperatures.

Working Procedure:
1. Sample preparation
   - The sample is weighed accurately
   - Put into a thimble (special extraction container)
2. Extraction process:
   - Carried out using a Goldfish extractor
   - Organic solvent (usually petroleum ether) is continuously flowed
   - The process lasts for 4-6 hours at a certain temperature
3. Final stage:
   - Thimble containing residue is dried to constant weight
   - Calculation of fat content based on weight difference:
   * Initial weight of sample before extraction
   * Weight of residue after extraction and drying

Advantages of the Method
1. Solvent efficiency:
   - Closed circulation system allows solvent recovery up to 95%
   - Solvent can be reused after the purification process
2. Result accuracy:
   - Perfect extraction because the process is continuous
   - Can detect total fat including bound fractions
3. Practical aspects:
   - Suitable for routine analysis
   - Can handle multiple samples simultaneously

Calculation of fat content is as follows:

$$\text{Fat Content}(\%) = \frac{(\text{initial weight} + \text{filter paper weight}) - \text{final weight}}{\text{initial weight of sample}} \text{x}100\%$$

e. Carbohydrate Content Analysis by Difference (Nuri Andarwulan et al., 2014)

Carbohydrate content is usually given as total carbohydrates by difference, meaning that the content is obtained by subtracting 100% from % of other components (water, ash, fat and protein).

$$\text{Carbohydrate content (\%)} = 100\% - \% \text{ content (water + ash + fat + protein)}$$

f. Analysis Method

In this study, a Complete Randomized Design method was used with 5 replications. After the results of the proximate analysis were carried out using the method that has been described, a One-Way ANOVA analysis was carried out with Duncan's further test using IBM SPSS 27 software.

RESULT

Table 1 Average results of proximate test of *Litopenaus vannamei* head protein hydrolysate with rice bran flour mixture

Treatment	Testing				
	Water	Protein	Fat	Ash	Carbohydrate
0 Day	48%	24%	11%	6%	10%
5 Day	47%	29%	3%	7%	13%
10 Day	46%	22%	2%	8%	23%
15 Day	44%	20%	2%	8%	25%

From the average results of proximate testing covering Water, Protein, Fat, Ash, and Carbohydrate data, the results of water content decreased on each day of testing which on day 0 there were 48% to 44% on the last day, while for protein content, the results increased on day 5 while on days 10 and 15 there was a decrease from 29% to 20% the same thing happened to fat content, which decreased along with increasing days from 11% on day 0 to 2% on day 15, this is in contrast to the last two tests, namely ash content, which increased on day 0 there were 6%, and on day 15 there were 8% ash content, the same thing happened to the carbohydrate content test which on day 0 there were 10% carbohydrates then increased on day 15 to 25%. Discussion of the results of each test will be discussed in the next sub-chapter.

DISCUSSION

A. Water Content Analysis

In the analysis of water content, the results of significance P <0.05 were obtained, where with the difference in storage period there was a significant difference in water content and the best results were obtained at 15 days of storage. This decrease in water content was due to several factors, the first of which was the reduction in microbial activity in the protein hydrolysate component in line with previous studies which stated that water content will decrease over time in line with the decrease in microbial activity in the product, thus reducing the overall water content (Kowalska *et al.,* 2022; Sun *et al.,* 2016).

B. Protein Content

In the protein content test, the results obtained were P <0.05, which means that there is a significant difference in the protein content test results, and the results of further tests from this test obtained the best results on the 5th day of storage. On days 0 to 5, there was a high increase in protein levels. This is because protein aggregation occurs where various types of proteins combine to form more protein folds (Sharma & Luthra-Guptasarma, 2009). Furthermore, protein hydrolysis also occurs which can increase the overall protein content measurement (Xi et al., 2024). In testing after the 5th day, there was a significant decrease in the protein content test results. This is because protein degradation occurs due to enzymatic activity, which results in protein damage into smaller peptides and smaller amino acids (Chen *et al.,* 2024; Shukla *et al.,* 2015; Xu *et al.,* 2022).

C. Fat Content

In the analysis of fat content, the results of P <0.05 were obtained, which means that there is a significant difference in the results of the fat content test, and the results of further tests, the best results were obtained on day 0, this is due to fat oxidation which can reduce fat content

over time (Lorenzo *et al.,* 2014) fat also has characteristics that are easily rancid, plus if it interacts with water and microbes it becomes free fatty acids and glycerin which are accelerated by the enzymatic process carried out by microbes in protein hydrolysates (Al-Ismail *et al.,* 2007) in addition to the presence of microorganisms in protein hydrolysates where these microorganisms will metabolize fat which will degrade fat and produce off-flavor compounds that reduce fat content (González Hurtado *et al.,* 2014; Lorenzo *et al.,* 2014)

D. Ash Content

In the ash content analysis, the results of P <0.05 were obtained, which means that there was a significant difference in the ash content test results, and the results of further tests, the best results were obtained on day 0. It can be seen from the results obtained from the ash content test that there was an increase over time. This is because, due to microbial activity in protein hydrolysate, mineral decomposition occurs which can increase the ash content in the entire test (Xiao *et al.,* 2022) besides that in this process there is also an accumulation of inorganic components (Assa *et al.,* 2019)

E. Carbohydrate Content

In the analysis of carbohydrate levels, the results obtained were P <0.05, which means that there is a difference in the results of the carbohydrate level test and the results of further tests obtained the best results, namely on day 0. From the test results, an increase in carbohydrate levels was obtained along with the progress of the test, this is because there is a protein degradation process that occurs during the storage process which changes fat into free fatty acids and also glycerol (Al-Ismail *et al.,* 2007).

CONCLUSION

The results of this study indicate the potential of mixing rice bran flour with shrimp head protein hydrolysate, especially with the increase in protein levels in the mixture on the 5th day, this is very good for making food supplements that require high protein values, in addition, if left longer, the results of this mixture have a higher carbohydrate value, this is suitable as a tablet mixture so that it can be packaged in tablet form. The results of the characterization of the mixture of rice bran flour with shrimp head protein hydrolysate are expected to be a reference for further research so that food supplements can be produced that can increase the nutritional value of the Indonesian people.

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