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Paper Accepted for IJOO Paper Code: 67805

To: Suparmi <teten_58@yahoo.co.id>

Dear **Suparmi,**

Paper Code: 67805

We are very pleased to inform you that your paper **"Production and Characteristics of Rebon Shrimp (Mysis relicta) Protein Hydrolysate with Different Concentrations of Papain Enzymes"** is accepted by our Editor-in-chief for our journal **International Journal of Oceans and Oceanography (IJOO).** Please find attached copyright transfer form.

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Manuscript (2019)

Production and Characteristics of Rebon Shrimp (*Mysis relicta*) Protein Hydrolysate with Different Concentrations of Papain Enzymes

By:
Suparmi[1),] Harahap[1)] Nursyirwani[2)].Irwan Efendi[3)],Dewita[4)]
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Abstract

Rebon shrimp is one of the marine products with a very small size compared to other types of shrimp. Despite the fact that it is readily available and nutritionally rich, its usage has been limited, except that it is used as raw material for shrimp paste, cincalok and dried shrimp. Therefore, there is need to conduct more research on rebon shrimp in order to get more economically valuable products. The aim of this study is to produce rebon shrimp protein hydrolysates using an enzymatic process, with different concentrations of the papain enzyme.

The non-factorial completely randomized design was used in this experimental study with 3 treatment levels - addition of 5%, 10% and 15% concentrations of papain enzyme. Proximate analysis was conducted to determine its ash, protein, and fat content. Its protein analysis was also conducted to determine all the amino acids present. The results showed that papain enzyme at a concentration of 15% (b/v) was best for producing the rebon shrimp protein hydrolysate. Also, the chemical composition of of the protein hydrolysate produced include: ash content 3.41% (b / k), protein content 84.81% (b / k), and fat content 2.39% ash (b / k). The protein analysis resulted in 15 types of amino acid - aspartic acid, glutamic acid, serine, histidine, glycine, threonine, arginine, alanine, tyrosine, methionine, valine, phenylalanine, isoleucine, leucine and lysine. The glutamic acid has the highest amino acid level at 4.03% while histidine has the the lowest at 0.37%.

Keywords*: Amino acids, enzymes, hydrolysates, concentrations, Papain.*

INTRODUCTION

The fishing industry in Indonesia has numerous opportunities due to its geography which is majorly on the islands. Among the various shrimp types present, rebon stands out due to its huge presence and good prospects in the industry. In 2011, the amount of rebon shrimp caught was 3274.5 tons, this skyrocketed to 689,221.9 tons in 2012 and the revenue realized from it in that same year was 3,657,812,420 rupiahs (DKP Cilacap, 2012).

Rebon shrimp is generally used as raw material for producing paste, cincalok, dried shrimp, as well as animal feeds. Considering the fact that it has a fairly high protein content and a distinctive flavor, it is used in the production of protein hydrolysate products.

Generally, products from protein hydrolysate are made from proteins break down into simple peptides and amino acids through the process of hydrolysis by enzymes, acids or bases. However, hydrolysis through enzymes is the most effective due to the fact that it produces protein hydrolysates, which, according to Kristinsson (2007), do not damage certain amino acids like tryptophan and glutamine. The protease enzymes used in hydrolysis process are commercially available and an example is the papain enzyme, isolated from the sap of papaya plant (*Carica papaya*).

According to Ariyani (2003) and Hidayat (2005), several research works have been reported on the production of protein hydrolysates from various fishery products, however, there is none on the production of protein hydrolysates from rebon shrimp. Therefore, the aim of this study is to produce rebon shrimp protein hydrolysates using an enzymatic process, with different concentrations of papain enzyme. The non-factorial completely randomized design was used in this experimental study with 3 treatment levels - addition of 5%, 10% and 15% concentrations of the papain enzyme. Proximate analysis was conducted on the rebon shrimp protein hydrolysates to determine its ash, protein, and fat content. Its protein analysis was also conducted to determine all the amino acids present in it.

RESEARCH METHOD

Materials and Equipment

The materials used in this study include: rebon shrimp (*Mysis relicta*), papain enzyme, NaOH, HCl 0.02 M, H_2SO_4, H_3BO_3, chloroform solution, aquades indicators, aluminum foil, filter paper, tissue, label paper, polyethylene plastic, gloves, and masks. And the equipment include: centrifuges, pH meters, incubators, water baths, analytical scales, ovens, measuring cups, measuring flasks, blast furnaces, Erlenmeyer, drip pipettes, stainless blenders, mortars, desiccators, porcelain saucers and High Performance Liquid Chromatography (HPLC).

Research Method

The non-factorial completely randomized experimental design method with 3 treatment levels was employed in this experimental study. The treatment was the addition of different concentrations of the papain enzyme - 5%, 10%, and 15% papain enzyme. Also, parameters like the ash, protein and fat contents of the protein hydrolysate produced were analyzed, as well as its total its total amino acids.

Preparation

The rebon shrimp used in this study were freshly taken from a river in the city of Siapiapi in Rokan district. The shrimp was thorougly washed to remove any form of impurities on its body and then crushed with a mortar until a paste like substance was formed. This method of producing rebon shrimp protein hydrolysate is in accordance with the method of Karnila (2012). 300 grams per crushed sample of the rebon shrimp was weighed, distilled water 1: 1 (b / v), was added while stirring it and then allowed to homogenize for about 2 minutes. Then its pH is adjusted to 7.0, which is the optimal pH of the papain enzyme, by adding 1 M NaOH solution and 1 M HCl solution. The papain enzyme was added to the resultant mixture at three different concentrations of 5%, 10%, and 15%. Each mixture was kept in an incubator at a temperature of $60^{o}C$ for 24 hours and then boiled in a water bath at $85^{o}C$ for 15 minutes to activate the enzyme.

It was centrifuged and the liquid at the top, which is supernatant, was separated from the residuals by filtering. The supernatant is the rebon shrimp protein hydrolysate.

RESULTS AND DISCUSSION

Characteristics of Rebon Shrimp Protein Hydrolysate

The chemical compositions and the characteristics of the protein hydrolysate were observed to study the ash, protein and fat contents, as well as the total amino acidt.

Chemical Composition of the Rebon Shrimp Protein Hydrolysate.

The chemical composition of the protein hydrolysate was determined through the proximate analysis. The results showed its ash, protein, and fat contents as shown in Table 1

Table 1 - Chemical Composition of Rebon Shrimp Protein Hydrolysates with Different Papain Enzyme Concentrations

Treatment	Ash(BK)	Protein(BK)))	Fat
T_1	2.54_a	80.31_a	3.40_b
T_2	3.24_b	83.96_b	3.17_b
T_3	3.41_b	84.81_b	239_a

Description: T1 = Addition of 5% papain enzyme, T2 = Addition of 10% papain enzyme, and T3 = Addition of 15% papain enzyme.

Ash Content

Most food substances consist of about 96% organic matter and subjecting them to high temperature of about 600 °C burn off all the organic matter, leaving only the inorganic ones as ash. This contains various elements like Ca, Mg, Na, P, K, Fe, Mn and Cu. And according to Winarno (2008), ash is an indication of the mineral content of food when subjected to high temperature. Also, all these mineral elements are parts of the body-forming elements which helps the body to function well. The ash content of the rebon shrimp protein hydrolysate produced is as shown in Table 2.

Table 2 - The Average Value of Ash Content (% bb) of Rebon Shrimp Protein Hydrolysate with Different Papain Enzyme Concentrations

Treatment	Replication			average
	I	II	III	
T_1	2.53	2.66	2.42	2.54_a
T_2	3.10	3.26	3.34	3.24_b
T_3	3.39	3.43	3.41	3.41_b

Description: T1 = Addition of 5% papain enzyme, T2 = Addition of 10% papain enzyme, and T3 = Addition of 15% papain enzyme.

Considering the Table 2, the average ash content of the rebon shrimp protein hydrolysate was between 2.54 and 3.41%. The highest of 3.41% was found with the addition of 15% papain enzyme (T3) while the lowest, 2.54%, was found in the 5% (T1). The analysis of variance showed a significant difference between the protein hydrolysate and the ash content as F_{count} (64.05)> F_{table} (3.46) at 95% confidence level. Based on this, H_0 was rejected and honest significant difference (HSD) test was conducted and the results showed that the ash content with T_1 (5%) treatment level was significantly different from T_2 (10%) and T_3 (15%), however, there was no significant difference between T_2 (10%) and T_3 (15%) at 95% confidence level.

Also, the higher the concentration of the papain enzyme added to the protein hydrolysate, the higher ash content. This is caused by the resultant hydrolysis when NaOH and HCl was added to the protein hydrlysate in order achieve and maintain the optimum pH of 7.0. And according to Thiansilakul et al. (2007), increase in ash content is generally caused by the addition of compounds that can form salts during hydrolysis process. Hence, the addition of NaOH and HCl to take the experiment to the optimum pH resulted in the formation of mineral salts. More so, Gesualdo and Li-Chan (1999) reported that mixing acidic and alkaline compounds in protein hydrolysate solutions will result in the formation of salts, thereby increasing the ash content of the protein hydrolysates.

Protein Content

Protein is the most important component of protein hydrolysate, in terms of meeting the needs of animal protein, especially from fish. Also, the quality of hydrolysate product is determined to a large extent by its proteins content, which are essential in building up the body of living things as well as its functionalities. Vaclavik and Christian (2008) in a study conducted, reported that proteins consist of amino acid chains linked to peptide bonds and form a variety of complex structures in the body. The proteins content of the hydrolysate is shown in Table 3.

Table 3 - The Average Value of Protein (% bb) in Rebon Shrimp Protein Hydrolysate with Different Concentrations of Papain Enzyme.

Treatment	Replication			Average
	I	II	III	
T_1	80.67	80.31	79.94	80.31_a
T_2	82.86	85.05	83.96	83.96_b
T_3	83.96	85.42	85.05	84.81_b

Description: T1 = Addition of 5% papain enzyme, T2 = Addition of 10% papain enzyme, and T3 = Addition of 15% papain enzyme.

Table 3 shows that the average proteins content of the hydrolysate was between 80.31 and 84.81%. The highest of 84.81% was found with 15% papain enzyme (T3) while the lowest, 80.31%, was in the one with 5% papain enzyme (T1).

Also, the analysis of variance showed a significant difference between the protein hydrolysate and the protein content since F_{count} (26.95)> F_{table} (3.46) at 95% confidence level. Hence, H_0 is rejected and HSD test was conducted which showed that the protein content with T_1 (5%) treatment was significantly different from T_2 (10%) and T_3 (15%), however, there was no significant difference between treatments T_2 (10%) and T_3 (15%) at 95% confidence level.

More so, the higher the concentration of papain enzyme added to the protein hydrolysate, the higheer its protein content. This is because enzyme breaks down proteins to simpler elements like amino acid during hydrolysis and since enzymes are also proteins, more proteins are detected in the protein analysis. And according to Hasanlaniza (2010), the higher the concentration of protease in hydrolysis process, the more the nitrogen dissolved in fish protein hydrolysate. Furthermore, Iskandar and Desi (2009) reported that the higher the concentration of enzymes added to hydrolysis, the faster the speed of the reaction, but within certain limits, the hydrolysate obtained will be constant with increasing concentration of enzymes. This is because as the enzyme is continuously added, a time comes when it is no longer active thereby causing catalytic work depending on the concentration of the existing substrates. And according to Purbasari (2008), the increase in the protein content of hydrolysate products is caused during hydrolysis, in which insoluble proteins are converted into soluble hydrogen compounds which are further broken down to form simpler compounds that can easily be absorbed by the body such as amino acids.

Fat Content

Fat is a food type that functions as an energy source. And substances with low level of fat are more resistant to hydrolysis and lowers its chances of burning. And generally, hydrolysate products with low fat content have longer durability, better quality and are more stable in nature compared to those with high fat content. The fat content of the rebon shrimp protein hydrolysate is as shown in Table 4.

Table 4 - Average Value of Fat Content (%bb) in Rebon Shrimp Protein Hydrolysate with Different Concentrations of Papain Enzyme

Treatment	Replication			Average
	I	II	III	
T_1	3.50	3.45	3.24	3.40_b
T_2	3.26	3.17	3.09	3.17_b
T_3	2.19	2.33	2.64	2.39_a

Description: T1 = Addition of 5% papain enzyme, T2 = Addition of 10% papain enzyme, and T3 = Addition of 15% papain enzyme.

The average fat content of the protein hydrolysate as shown in Table 4 is between 2.39 and 3.40%. And the highest fat of 3.40% was found with 5% (T1) treatment level while the lowest, 2.39%, was found in the one with 15% (T3). The analysis of variance showed a significant difference between the protein hydrolysate and the fat content since F_{count} (412.55)> F_{table} (3.46) at 95% confidence level. Hence, H_0 is rejected and HSD was conducted which indicated that the fat content with treatment T3 (15%) was significantly different from treatment T2 (10%) and T1 (5%), however, there was no significant difference between T1 (5%) and T2 (10%) at the 95% confidence level.

The results showed that the higher the concentration of the papain enzyme, the lower the fat content. This is because the structure of fish tissue changes during hydrolysis such that fat can be removed with insoluble proteins through centrifugation. And according to Witono et al., (2015), a decrease in fat content of fish protein hydrolysate is caused by changes in the structure of fish tissue during enzymatic hydrolysis and it is usually very fast. Also, Purbasari (2008) reported that the low fat content of fish protein hydrolysate is caused by hydrolysis characterized with a very rapid change in the structure of fish tissue. On subjecting a thin part of fish muscle under an electron microscopic, it was observed that the myofibril protein has decreased drastically during hydrolysis, and the membrane muscle cells looks relatively resistant to damage. This is because these membranes tend to converge and form insoluble bubbles during hydrolysis, thereby resulting in the loss of lipid membranes.

In addition, Table 4 shows that the fat content of the protein hydrolysate is less than 5%. This is in line with the work of Roslanet al (2014). According to that research, several studies have reported the fat content of various protein hydrolysates to be less than 5%. Also, Purbasari (2008) stated that protein hydrolysate products with low fat content are generally more stable compared to those with high fat content. Furthermore, the low fat content of hydrolysate products can be used as ingredients for diet food especially those low fat content. It can as well be used in making baby food and as supplement in producing bread.

Amino Acid Composition

There are two main groups of amino acids: essential and non-essential amino acids. And according to Belitz et al., (2009), essential amino acids cannot be produced by the body, thus must be supplied through food. These include: valine, leucine, isoleucine, phenylalanine, tryptophan, methionine, threonine, histidine, lysine and arginine. The non-essential amino acids on the other hand, can be produced in the body and these include: glycine, alanine, proline, serine, cysteine, tyrosine, asparagine, glutamic acid, aspartic acid and glutamine. The amino acids constituents of the protein hydrolysate are shown in Table 5.

Table 5 - Amino Acid Composition of Rebon Shrimp Protein Hydrolysate

Amino acid	Total (%)
Essential amino acids	
Valine	1.22
Phenylalanine	1.10
Isoleucine	1.33
Histidine	0.37
Leucine	1.98
Threonine	1.00
Arginine	1.18
Lysine	1.42
Methionine	0.77
Non-essential amino acids	
Aspartic acid	2.87
Glutamic acid	4.03
Serine	0.95
Glycine	1.18
Alanine	1.62
Tyrosine	0.63

The 15 types of amino acids detected in the protein hydrolysate are shown in Table 5. There were 9 essential amino acids - valine, leucine, isoleucine, methionine, threonine, histidine, lysine, arginine and phenylalanine. While 6 non-essential amino acids were detected and these include: aspartic acid, glutamic acid, serine, glycine, alanine and tyrosine. This result showed that the protein hydrolysate contains almost all the available amino acids except tryptophan, proline, cysteine, asparagine and glutamine. And according to Cholifah (2014), a hydrolysis process which runs perfectly well can produce hydrolysates with 18 to 20 amino acids. Also, Kurniawanet al. (2012) reported that hydrolyzed proteins generally produce amino acids, but some proteins also produce other protein molecules that are still bound aside the amino acids.

Table 5 also show that glutamic acid is highest constituent of the protein hydrolysate with 4.03% composition. This is the most common amino acid in fish which determines its taste and flavor. And according to Ovissipour et al (2010), amino acids, like glutamic acid, aspartic acid, glycine and alanine, play important role in giving fish its flavor. Therefore, it is applied as *flavor enhancer*. And considering the fact it contains almost all the essential amino acids, it has the potential to be developed into providing essential amino acids in food products. Hidayat (2005) stated that hydrolysate products can be included in the menu of patients suffering from digestive disorders. And as regards the least available amino acid, which was histidine (0.37%), Almatsier (2006), suggested that it can be supplemented with proteins from other sources that have different amino acids since proteins have the capacity to complement each other.

CONCLUSIONS AND RECOMMENDATIONS

Conclusions

The addition of papain enzyme, at varying concentrations, to the rebon shrimp protein hydrolysate changed its the ash, protein, and fat contents. And based on the analysis, the best rebon shrimp protein hydrolysate was discovered with the addition of 15% papain enzyme (T3), with the following chemical composition: 3.41% ash content, 84.81% protein content and 2.39% fat content.

Also, the protein hydrolysate contained 15 amino acids which are aspartic acid, glutamic acid, serine, histidine, glycine, threonine, arginine, alanine, tyrosine, methionine, valine, phenylalanine, isoleucine, leucine and lysine. And while the amino acid with the highest percentage composition was glutamic acid with 4.03%, the lowest was histidine with 0.37%.

Recommendations

Considering the results of this experimental study, the authors suggest that:

1. 15% of papain enzyme should be used in producing rebon shrimp protein hydrolysate,
2. Further research should be conducted on the application of rebon shrimp protein hydrolysate in its usage as flavor ingredient and enhancer.

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