Fortification of Rebon Shrimp Protein Hydrolysate 
(*Acetes erythraeus*) in Sago Flour as a Nutritious Food

S. Suparmi*, T.Warningsih, D.Dahlia and S.W.Sidauruk
Department of Aquatic Product Technology, Faculty Marine and Fisheries Science, Universitas Riau
*Correspondence author : teten_58@yahoo.co.id

**Abstrak.** Rebon shrimp protein hydrolyzate as a non-conventional processed product has a high protein content which is the potential to be used to enrich the nutritional value of food ingredients such as sago flour. This study aims to determine the appropriate concentration of rebon shrimp protein hydrolyzate for enriching the nutritional value of sago flour. The addition of protein hydrolysate used 3 levels of concentration, those were 10% (F1), 15% (F2) and 20% (F3). The best treatment is F3. Fortification of rebon shrimp protein hydrolysate in different amounts has an effect on the proximate value of sago flour, the best treatment is a concentration of 20%, with a water content of 10.49%, 4.85% ash, 24.56% protein and levels. fat 1.76%. Hedonic test indicated the taste value of 8.7, aroma 8.6, appearance value 8.7 and texture value 8.6. The content of amino acids is 17 types with 9 essential amino acid. The essential amino acids are histidine, arginine, threonine, valine, methionine, isoleucine, leucine, phenylalanine and lysine. The 8 types of non-essential amino acids are aspartic acid, glutamic acid, serine, glycine, alanine, proline, tyrosine and cysteine. The highest essential amino acid content was leucine at 2.75% and the highest non-essential amino acid content was glutamic acid at 4.52%. Based on the research results, it can be suggested for the enrichment of the nutritional value of sago flour to add 20% protein hydrolyzate of rebon shrimp and it can be used as a nutritious food.

**1. Introduction**

Indonesia as a tropical country that is very rich in biological resources and a variety of culinary cultures has used a lot of natural resources to get the taste of the taste, and there are many natural ingredients that have the potential to be very diverse food ingredients. There are still many natural sources, especially marine products that have not been explored, the opportunities and challenges for the development of food made from marine products are very appropriate because of the climate, culture and potential that are very supportive.

Rebon shrimp contains protein, calcium and iron. Besides, rebon shrimp also has the advantage that all parts of its body can be consumed, so it is necessary to have direct exploration to find out its potential so that it can be produced and utilized optimally. One of the technologies is to use enzymes to hydrolyze it to get the rebon shrimp protein hydrolysate which contains amino acids with various peptides and nucleotides. Fish and shrimp protein hydrolysate is a liquid product made with proteolytic enzymes with the final result in the form of a mixture of protein components, used as a fortification material to enrich the nutritional value of food products [1].

According [2], the function of protein hydrolyzate can be used as an ingredient to add nutritional value, as a flavoring and source of amino acids or it can also be used for treatment, namely as a diet for digestive sufferers. From the research results [3], that the protein hydrolyzate from the byproduct of fish processing using two types of enzymes, namely alkalase enzymes and flavourzyme enzymes, the best results are obtained from...
alkalase enzymes, because fish protein hydrolyzates are produced in large quantities and have large solubility, emulsifier and lathering characteristics.

Food products today are demanded not only to fulfill conventional nutritional needs and satisfy tastes but also as a guardian of health and also body fitness. Observations of experts show that active physiological activity in food can affect the health status of consumers [4]. According to Wijaya 2008, this phenomenon of course provides new challenges as well as opportunities for food technologists in developing their products. As with sago food, which has high starch content but low protein content, it is necessary to make an effort to increase its nutritional content.

The tendency at this time to use synthetic additives in food, so that it will cause insecurity for human consumption. Rebon shrimp, which are fundamentally rich in nutritional content but not yet maximized, have the potential to be used as a natural protein source and can be used in products with low protein content such as sago starch. There are many opportunities to produce nutritious food formulations sourced from nature and untouched local wisdom, therefore research on the hydrolyzate fortification of rebon shrimp protein in sago flour as a nutritious food, needs to be done to add and explore the existing potential.

2. Materials and Methods

2.1. Materials and Tools

The raw materials of this research were rebon shrimp, papain enzymes and sago. While the materials used for analysis are saturated toluene solution, absolute alcohol p.a. luff schoorl, chemicals for protein and amino acid analysis, NaOH, TCA, phosphate buffer, Mix-lowry (Na2CO3, CuSO4, NaKtart), TBA reagent (thioburic acid) HCL, Isobutanol and ethanol. The tools used in this study were stainless blender, Yenaco YC-1180 centrifuge model and tube, Roy Spectronic 21 D Melton and its cuvette, Ph meter, magnetic stirrer, vortexthermoliyne type 160, water bath, analytical balance, heater gerhardt, spatula, vacuum, oven, distillation, biuret and film bottle.

2.2. Research methods

Preparation of shrimp protein hydrolyzate [5]. Fresh rebon shrimp as much as 500 grams is cleaned and washed with running water, then crushed, then 500 mL of distilled water is added with a ratio of 1: 1 (w / v) and stirred until homogeneous, then heated at 60°C for 15 minutes aims to activate the shrimp enzyme. rebon. Then the optimum pH was adjusted using 0.5 N NaOH to reach a pH of 7.0. After that, the enzyme papain was added with an amount of 15% by weight of rebon shrimp. Furthermore, to optimize the work of the enzyme, incubation was carried out at 60°C for 4 hours. After the hydrolysis process is complete, it is heated using a water bath at 85°C for 15 minutes, to separate the liquid and solid phases by centrifugation at 15,000 g for 15 minutes. The supernatant obtained was dried by Freeze drying, the result obtained was shrimp protein hydrolyzate.

2.3. Preparation of Fortified Sago Flour

The rebon shrimp protein hydrolyzate produced is fortified with sago flour and processed into instant sago, at this stage the design used is a completely randomized design with one factor, the addition of protein hydrolysate used 3 levels of concentration, those were 10% (F1), 15% (F2) and 20% (F3). The proposed mathematical model according to the design according [6], the material formulation in making fortified sago flour can be seen in Table 1.
Table 1. Sago flour formulation with the addition of rebon shrimp hydrolyzate

| Ingredient                      | F1 (10%) | F2 (15%) | F3 (20%) |
|---------------------------------|----------|----------|----------|
| Sago Starch (g)                 | 500      | 500      | 500      |
| Water (mL)                      | 1.5      | 1.5      | 1.5      |
| Skim (g)                        | 10       | 10       | 10       |
| Sugar (g)                       | 15       | 15       | 15       |
| Rebon shrimp protein hydrolyzate (%)) | 10       | 15       | 20       |

The parameters observed were proximate values, namely moisture, ash, protein and fat content, hedonic test. The best results from the formulation are then analyzed for the amino acid content.

3. Research Result

3.1. Proximate Analysis

Data from proximate analysis of protein hydrolyzate of rebon shrimp can be seen in Table 2.

Table 2. Proximate analysis of sago fortified with protein hydrolyzate of rebon shrimp

| Proximate   | F1 (10%) | F2 (15%) | F3 (20%) |
|-------------|----------|----------|----------|
| Moisture (%)| 9.47     | 9.58     | 10.49    |
| Ash (%)     | 3.86     | 3.78     | 4.85     |
| Protein (%) | 17.40    | 20.32    | 24.76    |
| Fat (%)     | 1.91     | 1.74     | 1.76     |

Based on the results of the study, it is known that the greater the concentration of protein hydrolyzate of rebon shrimp added, the greater the water content of sago flour, but the average water content value is still acceptable because the maximum standard based on SNI01-3709-1995 is 11%. The high protein content of the product, especially in sago flour fortified with 20% rebon shrimp protein hydrolyzate, is due to the influence of the protein content contained in the hydrolyzate. According to the research results presented [7], The protein content in protein hydrolyzate products, protein concentrates and protein isolates is high, this is because when the hydrolysis process occurs there is a change in insoluble protein into soluble nitrogen compounds, then decomposed to form simple peptide compounds, namely amino acids. The increase in relative protein content occurs due to a reduction in the fat content of fish protein hydrolyzates, due to the hydrolysis process, the presence of dissolved membranes so that the fat structure is reduced [8].

Fat contained in non-conventional processed products (hydrolyzates, concentrates, and protein isolates) of rebon shrimp is lower, this is due to the reduction in fat content in the hydrolysis process. From the research results [8], in the hydrolysis process, the protein content will increase while the fat content will decrease because during the hydrolysis process, the tissue membrane breaks down.

According [9], The amount of ash content of a processed product is different due to the addition of compounds that can form salt during the hydrolysis process. The addition of NaOH and HCl compounds to adjust to the optimum pH conditions causes the formation of mineral salts [8], stated that the mixing of acidic and alkaline compounds in a protein hydrolysis solution will cause the formation of salts, this can affect the amount of ash content, especially in protein hydrolysis.
3.2. Hedonic Test

The hedonic test results of fortified sago flour with protein hydrolyzate of rebon shrimp with a concentration of 0%, 19%, 15% and 20%, it can be seen that the highest average hedonic value is at a concentration of 20%, namely taste (8.7), aroma (8.6), appearance (8.7) and texture (8.6) which means that the panelists really like it with the characteristic savory taste, distinctive aroma of sago flour, yellowish appearance and fine texture.

| Concentration (%) | Panelists | Taste Value | Aroma Value | Appearance Value | Texture Value |
|-------------------|-----------|-------------|-------------|------------------|--------------|
| 0                 | 80        | 4.5± 0.2    | 3.2± 0.3    | 3.0± 0.2         | 3.4± 0.2     |
| 10                | 80        | 5.5± 0.2    | 5.0± 0.2    | 5.4± 0.3         | 6.5± 0.2     |
| 15                | 80        | 7.0± 0.2    | 6.5± 0.3    | 7.3± 0.2         | 6.6± 0.1     |
| 20                | 80        | 8.7± 0.1    | 8.6± 0.1    | 8.7± 0.2         | 8.6± 0.1     |

Sago flour which is fortified with a protein hydrolyzate concentration of 20% is the most preferred by consumers, this is due to the greater amount of concentration so that the number of components it contains is also greater than other concentrations, so that it has an effect on taste, aroma, appearance, and texture. The difference in panelist acceptance, according [10], due to the glutamic acid content of the product. The hedonic test is a test to determine the level of acceptance by panelists like it or not on a food product [11].

The sensitivity to taste varies depending on the substance being tested [12], that the relationship between the amount of impression obtained and the concentration of a material is directly proportional, the higher the concentration of the material, the higher the magnitude of the impression.

As explained [13] that glutamic acid can stimulate several types of nerves in the human tongue, glutamic acid and aspartic acid give seafood a taste, but in the form of sodium salt, which is MSG, it will give the taste of umami and most components important in the formation of flavors in seafood. In general, the most common amino acid content in marine mollusks are glutamic acid, aspartic acid, glycine and alanine [14]. Furthermore, according to [15], aroma is a smell sensation that arises due to compound stimulation.

3.3. Amino Acid Content

The amino acids contained in sago flour fortified with 20% concentration of rebon shrimp protein hydrolyzate are 17 types of amino acids, with a composition of 9 types of essential amino acids, namely histidine, arginine, threonine, valine, methionine, isoleucine, leucine, phenylalanine and lysine, and 8 types. non-essential amino acid content. This can be seen in Table 4.
| No | Amino acid         | Amount (%) |
|----|-------------------|------------|
| 1  | Aspartic Acid     | 2.51       |
| 2  | Glutamic Acid     | 4.52       |
| 3  | Serine            | 1.74       |
| 4  | Glycine           | 1.56       |
| 5  | Histidine         | 2.43       |
| 6  | Arginine          | 1.52       |
| 7  | Threonine         | 1.09       |
| 8  | Alanine           | 1.76       |
| 9  | Proline           | 1.57       |
| 10 | Tyrosine          | 1.02       |
| 11 | Valine            | 1.56       |
| 12 | Methionine        | 1.14       |
| 13 | Cysteine          | 0.78       |
| 14 | Iso-leucine       | 0.86       |
| 15 | Leucine           | 2.75       |
| 16 | Phenyl Alanine    | 1.03       |
| 17 | Lysine            | 1.30       |

Amino acid is an organic component containing amino and carboxyl groups. Not all amino acids can be made in the human body, when viewed in terms of their formation, amino acids are divided into two groups, namely exogenous amino acids and endogenous amino acids. Exogenous amino acids are also called essential amino acids and endogenous amino acids are also called nonessential amino acids. Essential amino acids are amino acids that cannot be made in the body and must be obtained from food sources of protein, also called exogenous amino acids, while non-essential amino acids are amino acids that can be made in the body [16]. Amino acids are often referred to and known as building substances which are the end product of protein metabolism.

The difference in amino acid content in the rebon shrimp product in each treatment can occur due to differences in processing methods, fishing season and where the organisms live [17]. The results of research on rebon shrimp products, it is known that there are 15 types of amino acids, consisting of 5 types of non-essential amino acids, namely aspartic acid, glutamic acid, serine, glycine, tyrosine, and 10 types of essential amino acids, namely histidine, arginine, threonine, valine, alanine, methionine, isoleucine, leucine, phenylalanine, and lysine. In accordance with the results of research [18], most of these amino acids were detected in fishery commodities.

4. Conclusions and Recommendations
The protein hydrolyzate fortification of rebon shrimp in different amounts affected the proximate value of sago flour. The best treatment was 20% concentration with 10.49% air content, 4.85% ash, 24.56% protein and 1.76% fat content. Hedonic test with a taste value of 8.7, aroma 8.6, visual value 8.7 and texture value 8.6. The content of 17 types of amino acids with details of 9 essential amino acids and 8 types of non-essential amino acids. The highest essential amino acid content was 2.75% and the highest non-essential amino acid content was glutamic acid at 4.52%. Based on the research results, further research can be
carried out, namely determining the type of packaging, packaging design and estimated expiration period.

References

[1] Wijaya CH. 2008. Formulasi permen fungsional Asia Pacific food Industry. 2(6):49-52.

[2] Wergedahl, H., Liaset, B., Gudbrandsen, O.A., Lied, E., Espe, M., Muna, Z., 2004 Fish protein hydrolysate reduces plasma total cholesterol, increases the proportion of HDL cholesterol and lowers acyl-CoA: Cholesterol acyltransferase activity in liver of Zucker rats. Journal of Nutrition 134:1320-1327.

[3] Muzaifa,M, Safriani,N.Zakaria,F. 2012. Production of protein hydrolysates form fish by product prepared by enzymatic hydrolysis. J. Aquaculture,Aquarium,Conservation & Legislation. Internasional Juornal of the Bioflux society

[4] Ohama H, Ikeda H, Moriyama H. Health foods and foods with claims in Japan. Toxicology. 2006. 221:95-11.

[5] Suparmi, Amrizal, and Dahlia, 2017. Fortification of Rebon Shrimp Protein Hydrolysis (Mysis relicta) As a superior product of the Riau Coastal Region. Higher Education Excellence Research, LPPM UNRI.

[6] Gasperz, V. 1991. Experimental Design Methods. Penerbit CV, Armico. Bandung.

[7] Purbasari, D., 2008. Production and Characterization of Protein Hydrolysates from Mas Ngur’s Shells (Atactodea striata) [Skripsi]. Outcome Technology Study Program Fisheries, Faculty of Fisheries and Marine Sciences, Agricultural Institute Bogor, Bogor.

[8] Thiansilakul, Y., Benjakul, S., dan Shahidi, F., 2007. Compositions, functional properties, and antioxidative activity of protein hydrolysates prepared from round scad (Decapterus maruadsi), J. Food Chemistry, 103 :1385–1394.

[9] Karim FA, Swastawati F, Anggo AD. 2014. The effect of different raw materials on glutamate content in shrimp paste. Jurnal Pengolahan dan Bioteknologi Hasil Perikanan. 3 (4): 51-58.

[10] Thariq, A. S., Swastawati, F., and Surti, T., 2014. Effect of Difference Concentration of Salt in Peda Fish (Rastrelliger negletus) to the Content of Glutamic Acid Giving Savory Taste (Umami), J. Pengolahan dan Bioteknologi Hasil Perikanan, 3 (3) : 104–111.

[11] Monfatmanaba, K. Y. 2009. Test the flavor range threshold. Ministry of Health of the Republic Indonesia. Health Polytechnic of Mataram.

[12] Uju, T., Nurhayati, B. Ibrahim, W. Trilaksani & M. Siburian. 2009. Characterization and recovery of protein from minced fish washing water using reverse osmosis membranes. Jurnal Pengolahan Hasil Perikanan Indonesia. 12 (2) :115-127.

[13] Derby CD, Kicklighter CE, Jhonson PM, Zang X. 2007. Chemical composition of inks of diverse marine molluscs suggests convergent chemical defenses. Journal Chemical Ecology 33(3): 1105-1113.

[14] Astuti, E. 2006. Instant Tiwul Nutrifiction with Egg Flour (Study and Protein Content and Organoletic Properties. Tesis. UMS. Semarang.

[15] Winarno F.G. 2004. Food Chemistry and Nutrition. Jakarta: Gramedia Pustaka Utama.

[16] Okozumi, M., dan Fujii, T., 2000. Nutritional and Functional Properties of
Squid and Cuttlefish. Tokyo University of Fisheries, Japan.

[18] Steed, R. 2010. Analysis of amino acids by HPLC. https://www.agilent.com/cs/library/slideshow/Public/Amino%20Acid%20Analysis_062410_Rita%20Steed.pdf