Study of Chemical Compound Content and Analysis of Glycoprotein Pokea Shell (*Batissa Violacea Celebensis Martens 1897*) from Southeast Sulawesi Province as Immunomodulators

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Abstract

Pokea shell (*Batissa violacea celebensis Marten 1897*) is a bivalve species originating from the Pohara River of Konawe Regency, Southeast Sulawesi. Water stew of meat shell that has never been used, it actually contains glycoprotein compound that can act as immunomodulatory agents or as antitumor. The purpose of this research is to process, and analyzes the extract results of chemical compounds on fresh, boiled and dried pokea shell, and test the effectiveness of pokea crude extracts by extracting glycoprotein, and subsequently can be made into dosage as a crude extract that is made as a food supplement (supplement food). Extraction of glycoprotein from shell has been done, by boiling the shell for 30 minutes and 60 minutes. The amino acid composition of the glycoprotein was analyzed by FTIR. The results showed that the result of glycoprotein with a boiling time of 30 minutes is higher than 60 minutes of boiling time. The glycoprotein content rendemen of pokea shell with 30 minutes boiling time is 4,42 gram, and 2,7 gram of 60 minutes of boiling time. It can be concluded that the extract of glycoprotein of pokea shell meat done for 30 minutes resulted in more amino acid variety than for 60 minutes. Further studies are needed to determine the levels and composition of the glycoprotein carbohydrates, it is important to know about the health benefits of each type of carbohydrate contained in the glycoprotein when used as food supplements.

Key words: Amino acids, Batissa Violacea glycoprotein, immunomodulators, Pokea shell

Introduction

Pokea shell (*Batissa violacea celebensis Marten 1897*) is a bivalve species of the Corbiculidae Family found in the Pohara River of Konawe Regency, Southeast Sulawesi (Bahtiar 2005). Pokea shell is usually traded in fresh form intact, fresh peeled, and satay. During processing, the nutrient content of a substance can be lost or damaged by sensitivity to heat, pH, oxygen, light, or combination of several factors (Harris 1988). Pokea shell is used by the local community, which is consumed daily by boiling. This is thought to affect the nutritional content. Many studies have proven that pokea shell is empirically believed to cure various diseases such as jaundice, malaria, asthma, lowering blood pressure and fever. The disease occurs due to infection by strange materials and microorganisms. When the body is infected with microorganisms, the body responds with macrophage and neutrophil activity mechanisms. In this case the oxidase and oxygenase enzymes will form various free radical compounds and reactive oxygen compounds, including hypochloridic acid (HOCl) which will attack and destroy viruses and bacteria. Radical compounds are also very dangerous because of the potential to attack the body's cells. If this is not controlled, it will trigger the emergence of various chronic diseases (Winarsi 2007).

Shell can live in clean and polluted environments. When living in a polluted aquatic environment it will have a specific body defense system including the fight against substances that are toxic and carcinogenic. Shells contain glycoprotein compounds that can act as immunomodulatory substances with high activity. Glycoprotein is complex compounds between

proteins with a covalently bonded oligosaccharide (glikan) chain. Glycoprotein is present in the human body, animals, viruses, bacteria, fungi and plants with various functions, for example as: structural molecules, lubricants and protective materials, transporting molecules (vitamins, lipids, minerals and trace elements), immunologic molecules, hormones, enzymes, cell/cell recognition, lectins and antifreeze substance. Glycoprotein molecular weight ranges from 15,000 to more than 1,000,000.

Many cases of disease are as result of the occurrence of irregularities or metabolic disorders in the receptor system on the cell membrane. Glycoprotein as one component of cell membrane plays an important role in maintaining normal metabolic conditions in the cell membrane. Damage to the cell membrane system may occur due to the fraction of proteins or carbohydrates undergoing mutations or damage by chemicals or viruses thus causing the reaction signals to process of metabolism cannot run smoothly or become disturbed. The next consequence is arising discomfort in the body that is perceived as a disorder or disease.

The content of glycoprotein in shell meat is estimated to be about 0.5% so it is not possible for us to get the glycoprotein from the shell directly. Therefore, this study was conducted to obtain glycoprotein from the shell meat. Therefore, the study in this research is considered very important to be done for efforts to increase the added value of pokea shell boiled water wasted through the production of the preparation as supplement food (supplement food). Based on this background, thus in this study conducted a study of chemical compound content and glycoprotein extraction and analysis of Glycoprotein amino acid composition from Pokea shell species taken from fishermen in Pohara River.

Method

INGREDIENTS. The material used is pokea shell, obtained from fishermen in Pohara river Konae regency of Southeast Sulawesi Province. Sample of fresh shell removed his skin, then shell is washed, ground, and stored in a tightly sealed container. If the chemical analysis cannot be done directly, then the sample is stored in a freezer.

METHOD. Chemical analysis. chemistry analysis including drying shrinkage, crude protein content (total nitrogen), and non protein nitrogen (NPN) done levels to the fresh shell samples. The shrinkage drying were determined by gravimetric method, crude protein content and NPN by Kjeldahl method.

Glycoprotein extraction. Glycoprotein extraction used Sasaki et al. method with slight modifications. A total of 500 grams of shell meat in 100 ml of 10% NaCl solution is boiled at 100 ° C, each for 30 minutes and 60 minutes. The hot solution is then filtered through Whatman's no. 1, the obtained filtrate was allowed to cool at room temperature, then stored in the refrigerator for 1 hour. Furthermore, ethanol is added to the cold filtrate (ratio 1: 2, v / v) so that the glycoprotein deposits occur. To complete the precipitation, the solution was centrifuged at 12,000 rpm at 4 C for 10 min. The filtrate was removed and the resulting precipitate was dissolved in 0.01 M phosphate buffer pH 7.4, and then purified by passing through the Sephadex G-100 chromatography column to remove salts and filter the proteins with molecular weight between 5,000-10,000. The obtained filtrate is freeze drying at -40 ° C, 200 × 10 mbar pressure. The extract of glycoprotein obtained in powder form, then weighed and analyzed the water content and amino acid content.

Amino acids analysis. The extract of glycoprotein obtained, then analyzed its amino acid content using FTIR (Fourier transsform Infrared)

Result

Morphometric and Rendement Measurements

Morphometrics of pokea shells

Measurement of morfometric of pokea shells was conducted on 104 samples. These measurements include intact weight, meat weight, shell weight, shell length and shell width to determine the yield. The average results of morphometric measurements can be seen in Table 1.

Table 1. Results of morphometric measurement of pokea shells

Parameter	Big size (n = 15)	Medium size (n = 45)	Small size (n = 44)
Weight intact (g)	73,47 ± 18,39	23,89 ± 10,79	6,08 ± 1,46
Weight of meat (g)	18,43 ± 7,36	6,11 ± 3,47	1,48 ± 0,46

Shell Weight (g)	37,73 ± 9,35	11,71 ± 5,54	3,09 ± 0,79
Liquid weight (g)	17,31 ± 3,79	6,07 ± 2,16	1,51 ± 0,41
Shell width (mm)	71,59 ± 6,72	48,85 ± 7,72	31,83 ± 3,31
Shell length (mm)	57,07 ± 4,90	39,63 ± 5,91	25,59 ± 2,19

The results of morphometric measurements on pokea shells, indicating diversity of sizes ranging from large, medium, and small. This is in accordance with Bahtiar report (2005) that the pattern of pokea shell population growth is relatively fast to slow due to the influence of sand mining activities and the removal of pokea shells. The Pokea shells meat multi-storey extraction can be seen in table 2.

Table 2. Pokea shells meat multi-storey extraction

Treatment	Extract Rendement
Pokea Fresh Shells	1,6984%
Pokea Rebus Shells	3,638%
Pokea dried shells	6,2328%

Meat pokea shells rendemen. Rendement is the percentage between the weight of a portion which can be utilized compared to the weight of the whole material. The part commonly used by the community as food is the meat. Based on the measurement results pokea shells meat rendemen is an average of 27,8%.

Shrinkage drying. A shrinkage drying analysis of a food product is required to calculate the level of the compound / substance in units of dried weight. Wet marine products typically contain moisture content of 70-85%. The results of proximate analysis on pokea meat include protein, fat, ash, and water. The proximate composition of fresh, boiled, and dried pokea meat can be seen in Table 3.

Table 3. Proximate composition (% w/w dry weight) of fresh, boiled, and dry pokea shells

Proksimat Composition	Fresh	Boiled	Dry
Air Water	85,3279%	71,9222%	32,8539%
Ash	0,7637%	1,2430%	3,4967%
Fat	1,0039%	2,7752%	12,367%
Protein	9,9722%	19,2262%	61,9343%
Carbohydrate	2,9323%	4,8334%	1.7331%

Identification of glycoprotein. To ensure that the extract obtained is true glycoprotein means to show positive reactions containing proteins and carbohydrates. Therefore, the identification is done to the glycoprotein extract obtained. The identification of glycoprotein was performed by color reactions using biuret reactor for the protein test and Molisch reactor for carbohydrate test. Based on the results of the identification can be concluded that the extract is true glycoprotein because it proved to give positive reaction results contain protein and carbohydrates. The results of identification are presented in Table 4.

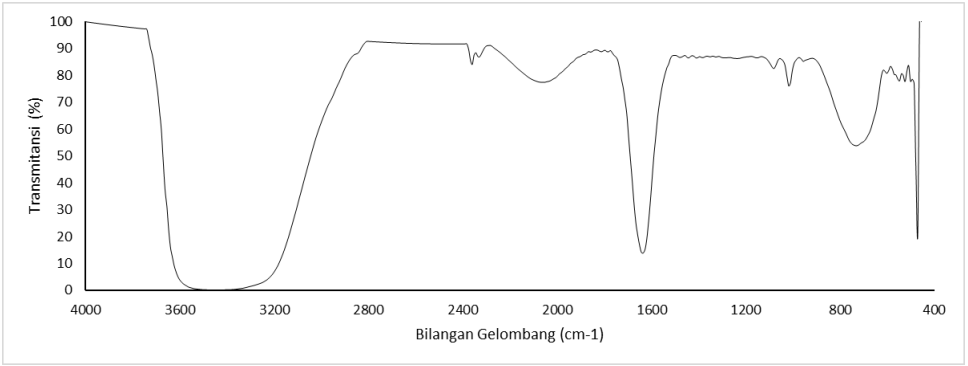
Table 4. The results of glycoprotein identification to extract from fresh samples of Pokea shells according to the duration of boiling

No.	Type Test	Terms	Fresh		Boiled		Dry	
			30 minute	60 minute	30 minute	60 minute	30 minute	60 minute
1.	Biuret Test							

	+ NaOH 10%	color	color	color	color	color	color	color
	+ $CuSO_4$ 1%	purple	purple	purple	purple	purple	purple	purple
2.	Molish Test							
	+ Naftol/E_tOH	circlet	circlet	circlet	circlet	circlet	circlet	circlet
	+ H_2SO_4 concentrated	purple	purple	purple	purple	purple	purple	purple

Glycoprotein content. Analysis of glycoprotein content was performed to the shell meat with two different treatments to determine the effect of boiling duration to glycoprotein level in shell meat. The analysis result of glycoprotein level rendemen for pokea shell with 30 minute boiling time is 4,42 gram, while with boiling time 60 minutes of pokea shell is 2,47 gram, it showed that the shorter boiling time produces higher glycoprotein levels. This is presumably because the shorter of boiling time, a little glycoprotein bonds are disconnected from the shell meat net so that it eventually becomes soluble.

Analysis of glycoprotein amino acids. In chromatogram FTIR results of the analysis of the sample appears only 16 peaks of amino acids. Thus, on the glycoprotein from the shell analyzed only 16 amino acids can be detected. The analysis results are presented in Figure 1.



wave number (cm^{-1})

Figure 1. Chromatogram FTIR results in boiling time 30 minutes

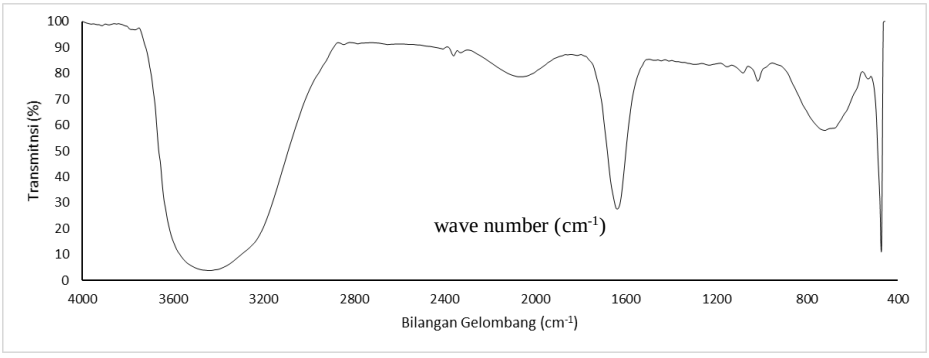


Figure 2. Chromatogram FTIR results in boiling time 60 minutes

The amino acids contained in both glycoprotein samples can be seen in Table 5.

Table 5. Amino acids contained in both glycoprotein samples

Amino Acids	Wavelength
Cys	1716-1788
Glu	1712-1788
Asn	1677-1678
Arg	1672-1673

His	1575-1631
Lys	1626-1629
Tyr	1600-1621
Asp	1574-1579
Glu	1556-1560
Lys	1526-1507
Trp	1509-1496
Phe	1494
Pro	1400-1465
Gln	1410
Ser	940-983
Thr	1075-1150

For quantitative calculations, it is done by comparing the peak area of each amino acid produced from the sample with that produced from the standard FTIR. The results showed that the boiling amino acid level for 30 minutes was higher than the boiling result for 60 minutes, but the content increase is not linear because there are 3 times, 2 times, and even the level is almost the same between the two boiling time. The differences may be caused by: (1) amino acid damage during the extraction process (boiling) at a temperature of 90-100 °C; (2) the use of centrifuges at 12.000 rpm makes the protein structure damaged. To determine the presence/absence of significant differences between the amino acid level produced by boiling for 30 minutes and 60 minutes of each shell meat, then a statistical analysis was performed using the t-test. The t-test results generally showed t-count > t-tables, and fewer t-counts that are smaller than t-tables. This shows a significant difference between the amount of amino acids produced by boiling for 30 minutes and 60 minutes.

The results showed that the amount of amino acid produced at boiling for 30 minutes was more than boiling for 60 minutes. Thus the isolation of glycoprotein by boiling time for 30 minutes was better variety than boiling for 60 minutes, and the results showed a significant difference.

Conclusion

The extract of glycoprotein from pokea shell meat made for 30 minutes resulted in more glycoprotein content than for 60 minutes. The extract of glycoprotein from pokea shell meat made for 30 minutes resulted in more amino acid variety than for 60 minutes.

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