Extraction and Characterization of Gelatin From Rabbitfish Skin (Siganus canaliculatus) with Enzymatic Method Using Bromelin Enzyme

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Abstract. Gelatin basically is a pure protein food ingredient, obtained from thermal denaturation of collagen from animals. Gelatin is used as a stabilizer, gelling agent, binder, thickener, emulsifier, adhesive, whipping agent, and edible coating food wrap. Protein levels in fish skin determines the amount of collagen contained in the skin tissue, so the Rabbitfish skin has a great potential as a source of collagen to be hydrolysed into gelatin. This study aimed to determine the characteristics of gelatin produced from Rabbitfish skin by enzymatic extraction as well as to determine the best treatment of combination of enzyme concentrations and extraction time. Gelatin production was carried out by hydrolysis using bromelain enzyme with concentration 1%, 1.5% and 2% with the extraction time of 2 hours, 4 hours, and 6 hours. The result obtained was gelatin with rendement ranging from 3,13% up to 5,83%, the water content ranging from 0,17% up to 3,56%, ash content 0,35% up to 3,65%, and protein levels ranged from 91% up to 94,72%. The chemical characteristics of gelatin from Rabbitfish skin have a yield of up to 6%, water content less than 6%, ash content less than 4% and protein content reaching 94%. The best treatment was obtained at 1% enzyme concentration and 4 hours extraction time, yielded gelatin with a protein content of 94,72%.

1. Introduction
Gelatin basically is a pure protein food ingredient, obtained from thermal denaturation of collagen from animals. Gelatin is a protein fraction that soluble in water through tertiary decomposition stage, secondary, and a certain extent the primary structure of collagen. Collagen derived from skin, white tissue and bones of animals. Gelatin is an important high molecular weight and hydrocolloid polypeptide, which has been proven popular in the general public and has been used in various food products, especially of gelling agents and viscosity.

Gelatin used as stabilizer, gelling agent, binder, thickener, emulsifier, adhesive, whipping agent, and edible food wrap. Gelatin used in jelly industry, meat industry, dairy industry and food supplements. In non-food industry, gelatin used in the pharmaceutical and cosmetic industries. In pharmaceutical industry, gelatin used in making of soft capsules, the shell capsules and tablets, in cosmetics industry as cosmetics component for shampoo and soap.

Large number of gelatin uses in various industries causes gelatin production increase every year. In 2010, production of gelatin world reached 326,000 tons in a year [15]. It is estimated around 59% of
The total gelatin produced worldwide is used in the food industry, 31% in the pharmaceutical industry, 2% in the photography industry, and about 8% is applied in other industries [10]. Recorded in Central Bureau of Statistics, Indonesia imported gelatin from abroad based on import data in 2013 is about 651,119 kg, in 2014 increase to 1,063,111 kg and increase in 2016 to 1,354,436 kg. According Karim [7] gelatin import market in the world (including Indonesia) raw material probably derived from pig skin (46%), cow (29.4%), beef bones (23.1%), and other sources (1.5%). So far, Indonesian gelatin needs are imported from countries like France, Japan, India, Brazil, Germany, China, Argentina and Australia [5].

The use of gelatin in Indonesia continues to increase, but many things that must be wary in this case. For example the issue of halal, mammal and bird or avian flu disease. In Islam, consuming food with pork content is not allowed. Gelatin from mammals and poultry is also at risk, such as mad cow disease (bovine spongiform encephalopathy), anthrax, and bird flu which can worsen health. Gelatin development from safer materials and guarantee halal status needs to be done.

One solution is the development of gelatin from marine products. Potential material as raw material for developing gelatin is rabbitsfish. Rabbitfish (Siganus canaliculatus) or rabbitfish fish has a large number of responsive aquatic products in South Sulawesi. Rabbitfish fish have quite high nutritional content. Skin is a body part of rabbitfish that can be used as an ingredient for gelatin. According Wahyuningtyas [18] rabbitsfish skin has 15.98% of protein content. Protein content of fish skin determines the amount of collagen contained in skin tissue, so rabbitsfish skin has a considerable opportunity as a source of hydrolyzed collagen to gelatin. Therefore, this study aimed to determine the characteristics of gelatin produced from rabbitsfish skin by enzymatic extraction and to determine the best treatment of a combination of enzyme concentrations with extraction time.

2. Methodology

2.1. Tool
The tools used in this study for preparation and extraction procedures gelatin, tools used is basins, knives, measuring cups, beakers, stopwatch, flask, thermometer, filter, oven, blender, water bath shaker, and analytical balance. And for the procedure of characterization of gelatin extracted, the tools used are kiln, porcelain cup, oven, and kjeldahl tool.

2.2. Materials
Materials used in this study is rabbitsfish skin, aquadest, bromelin enzyme from pineapple, pH indicator, HCl, NaOH, K₂SO₄, CuSO₄, H₃BO₃, H₂SO₄ and, Indikator BCG-MR.

2.3. Research Procedure
Research procedure includes sample preparation, hydrolysis with three types of enzyme concentration that are 1%, 1.5% and 2% with variation time extraction for 2 hours, 4 hours and 6 hours, testing the moisture content, ash content, and protein content.

2.3.1. Preparation and Making of rabbits fish Gelatin
Rabbitfish skin is cleaned from the rest of the meat and other impurities, rabbitfish skin cut into 1x2 cm size and washed with distilled water then cook for 2-10 seconds. The next stage is the hydrolysis of fish skin by using protease enzymes such as bromelain enzyme by treatment with (1%, 1.5% and 2%) for 2 hours. After the hydrolysis process is finished, the fish skin then washed with distilled water until neutral pH. Skin hydrolysis is then extracted by the addition of distilled water in ratio 1: 3 (Results hydrolysis: distilled) in a shaker water bath at 50°C for 2 hours, 4 hours and 6 hours. Extraction result filtered, and dried in an oven at a temperature of 45-50°C for 24 hours.
2.3.2. Water Content (AOAC, 1995)
Porcelain cup dried at 105°C for 1 hour. Then cooled and weighed. Plates contained sample is dried in oven at 105°C until its weight is constant.

2.3.3. Ash Content (AOAC, 1995)
Sample is previously dried in oven at 105°C until its weight is constant. Then put into furnace at 600°C until all the ingredients change color to gray, then the sample is weighed.

2.3.4. Rendement Calculation
The result is the weight of the extract divided by the sample weight and multiplied by 100%. Calculation of results needs to be done to determine the effectiveness of the enzyme. rendement shows the efficiency and effectiveness of the extraction process.

3. Results and Discussion

3.1. Rendement
Rendement is an important parameter in the process of making gelatin which is used to determine the effectiveness of the method used. The efficient and effective extraction rate of the raw materials used in making gelatin can be seen from the results produced. Large amount of rendement produced, shows the more efficient method of treatment used. Rendement was calculated by comparing the weight of the gelatin produced and the weight of raw material used. Rendement obtained in this study can be seen in the graphs below.

![Graph Calculation Results Rendement from RabbitsFish Skin Gelatin](image)

Figure 1. Graph Calculation Results Rendement from RabbitsFish Skin Gelatin

Treatment method used in this research is enzyme treatment concentration and long time of extraction. According to Ward & Courts [19] conversion of collagen into gelatin, affected by temperature, heating time and pH. Longer the time of extraction, rendement increased. This because the number of H⁺ ions hydrolyze more collagen, while the longer the extraction cause more biodegradable collagen into gelatin. Hydrogen bonds in tropokolagen denatured by H₂O molecules. Phase extraction causes the triple-helix molecule loses its stability and eventually break down into single chain gelatin. Hydrogen bonds in this tropokolagen denatured by H₂O molecules. This causes Rabbitfish triple-helix molecule loses its stability and eventually break down into single chain gelatin. Other than that, enzyme concentration also affect the yield of the resulting number, the higher concentration of enzyme used will increase peptide bonds that hydrolyzed. The yield resulting from the reaction between the substrate and enzyme concentrations are affected by the condition of enzyme. In the circumstances, the enzyme concentration increases while the fixed substrate concentration, concentration or number of molecules of enzyme is lower than number of substrate molecules to be catalyzed, so resulting product will be
proportional to the amount of substrate that is converted by enzymes into product. When the amount of enzyme increase, more substrate transformed into product so that once the amount of excess enzyme substrate exhausted yet. As a result, the addition of enzyme can not change the graph reaction to the concentration of the enzyme [1]. The results above show the difference in the yield produced by each treatment, but at a certain time, concentration and the resulting yield shows no straight line. So it can be said that in that state have been exhausted catalyzed substrate so that the graphs obtained showed no increase in yield results. The results obtained by the highest yield of 6.56% with a 2% treatment enzyme concentration and extraction time of 4 hours.

3.2 Water content

![Graph Calculation Results Moisture Fish Skin Gelatin Rabbitfish](image)

Water content is water contained in material that can be expressed based on the weight of wet and dry weight [17]. The water content can affect the appearance of texture, taste and food. Water content of gelatin will affect shelf life as closely related to the metabolic activities that occur during the gelatin stored as enzyme activity, microbial activity and chemical activity, the occurrence of rancidity and reactions non-enzymatic giving rise to changes in organoleptic properties and quality values [13]. The results gelatin water content measurement showed that the water content of fish skin gelatin from Rabbitfish obtained ranged 0.17% - 3.56% so that they meet the quality standard range of gelatin that is the maximum 16% (ISO 06-3735, 1995). The highest water levels by 3.56% obtained in the treatment of enzyme concentrations of 1.5% and long incubation of 2 hours, while the water levels low of 0.17% obtained at treatment 2% concentration and 4 hour incubation time. Low water content of the skin gelatin rabbitfish fish allegedly due to drying effect on the process of making this rabbitfish fish skin gelatin using 45-50°C temperature oven with commercial gelatin on commercial gelatin is usually used freeze dryer. Amiruldin M [2] states that, low water content will affect the quality of gelatin primarily on gelatin rancidity and color that is less bright.
3.3 Ash Content

Minerals are inorganic residue from the combustion of organic materials and usually these components are made up of calcium, sodium, iron, magnesium, and manganese. Ash has a white to gray color, smooth, and easy to dissolve. The purpose of the analysis was to determine the ash content in general that indicate minerals contained in the ingredients. Apriyantono et al [3] stated that the ash content of a food indicates the magnitude of the amount of minerals contained in the food material.

Gelatin ash content measurement results indicate that the ash content of fish skin gelatin rabbitfish obtained ranged between 0.35% - 1.65%. Ash content value rabbitfish fish skin gelatin suitable to ISO standards 06-3735, 1995 amounting to maximum 3.25%. The highest ash content of 1.65% obtained in the treatment of enzyme concentration of 1% and incubation time of 4 hours, while the lowest water content of 0.35% was obtained at 2% treatment enzyme concentration and incubation time of 4 hours. Determination of ash content is intended to determine the content of non-volatile components (components inorganic or mineral salts) that remained in the burning and annealed organic compounds [12]. More lower ash content of a material indicate the higher purity. High and low ash content of a material is partly due to the different mineral content in raw material sources and can also be influenced by the demineralization process at the time of manufacture [16].

3.4 Protein Levels

Minerals are inorganic residue from the combustion of organic materials and usually these components are made up of calcium, sodium, iron, magnesium, and manganese. Ash has a white to gray color, smooth, and easy to dissolve. The purpose of the analysis was to determine the ash content in general that indicate minerals contained in the ingredients. Apriyantono et al [3] stated that the ash content of a food indicates the magnitude of the amount of minerals contained in the food material.

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Protein content is one of parameters that determine quality of gelatin. Based on the test results of crude protein content, protein content of fish skin gelatin rabbitfish ranged from 92% to 94%. The highest protein content was found on enzymes 1% and 4 hours extraction time treatment which is 94.72% while the lowest protein content in the treatment of enzymes 1.5% for 6 hours extraction time which is 91.21%. This protein content is still high compared with levels of protein from various sources such as mackerel skin 23.13% [6], skin tuna amounted 24.63% [9], yellow tail fish skin by 17.87% [4] and sharkskin amounted to 27.73% [7]. Meanwhile, the protein content of Rabbitfish slightly lower than catfish skin gelatin. Proven by research Nasution et al [11] that made gelatin from the catfish skin with total protein amount of 97.71%. The difference between protein content is associated with the treatment. Enzyme concentration of 1% with 4 hour extraction provides the highest protein content. Testing for protein content meant to see the ability of gelatin in gel form. The higher levels of the protein, the ability to form a gel and viscosity increase. Based Santoso [14] said that protein in the gelatin greatly affect the formation of the gel. The higher levels of the protein will increase gelatin ability to form a gel and viscosity will increase. Based Santoso [14], protein in the gelatin greatly affect the formation of the gel.

4. Conclusion
Chemical characteristics of gelatin from rabbitfish skin has yielded up to 6%, the water content of <6%, ash content <4% and 94% protein content. The best treatment is obtained at a concentration of 1% and enzyme for 4 hours reaction with protein content of 94.72%.

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