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Extraction of catfish bone waste (*pangasius hypopthalmus*) by utilizing an organic liquid of pineapple peel waste (*anas comosus*) into gelatin

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Abstract. This study aims to determine how to extract the gelatin from catfish bones by using pineapple peel waste as a soaking material in the pre-treatment stage and examining the physicochemical properties of the gelatin produced. Kampung Patin in Kampar Regency, Riau Province, is one of the catfish cultivations in Indonesia. It is a center for producing catfish that produces fish bone waste. In this study, gelatin extraction will be undertaken in several stages: the preparation of pineapple waste liquid extract and gelatin extraction (pre-treatment and main extraction stages). Catfish bones will be soaked in 1:5 (m/v) pineapple waste liquid at the pre-treatment stage for 24 hours. While in the central extraction, ossein will be immersed in water at a temperature of 75°C for 5 hours. The analysis results for physicochemical characteristics of gelatin obtained include yield of 2.55, pH of 4.58, water content; 11.66%, ash content; 17.71%, crude fat content; 0.17%, protein content; 64.71%. It can be inferred that it meets the established gelatin quality standards from various physicochemical characteristics of gelatin in this study.

1. Introduction

The demand for gelatin is intensifying every year; gelatin from pork is the primary source on the market. In 2015, sources of gelatin worldwide were derived from pork skins 46%, cow skins 29.4%, beef and pork bones 23.1%, and other sources 1.5% (Gomez-Guillen *et al.*, 2009; Duconseille *et al.*, 2015). Only about 1% comes from other sources (Ahmad & Benjakul, 2011). Kampar Regency, Riau Province, is one of the centers of catfish cultivation in Indonesia. Catfish processed into fillets have a higher economic value (Kemendag RI, 2013). This processing produces waste of fish skin and bones. It causes problems for the environment since its utilization has not been maximized (Agustin, 2013). Fish bones can be a source of raw material for the manufacture of gelatin. It has economic value and can overcome the problem of waste caused to the environment at the same time.

Research (Mahmoodani, 2004) reveals that catfish bones produce gelatin with a higher concentration than gelatin from other fish bones. It is in line with research from (Oktaviani, 2017) which mentions gelatin from catfish (*Pangasius hypophthalmus*) products produce gelatin compared with commercial gelatin. (Zhang *et al.*, 2011) say that gelatin made from warm-water fish species has better physical characteristics than cold-water fishes. The necessary physical characteristics of gelatin include gel strength, texture, and viscosity (Badii & Howell, 2006; Ratnasari *et al.*, 2013).

The gelatin manufacture using organic materials has not been widely used. One of the natural ingredients can be utilized for the manufacture is found in pineapple waste. Pineapple contains citric



acid and bromelain enzymes have a high ability to break peptide bonds in proteins to convert collagen protein into gelatin (Nur & Rehalat, 2017). Utilizing pineapple waste for gelatin manufacture from fish bones, in addition to increasing value-added and minimizing agro-industrial wastes, can reduce the danger or risk of using chemicals. Pineapple waste liquid contains citric acid of 0.18-0.32% (Hajar et al., 2012).

On this occasion, the researchers would like to research the physicochemical properties of gelatin derived from the catfish bones (*Pangasius hypophthalmus*) taken from the Kampar area, precisely in Kampung Patin, which has the production of farming catfish in Riau. In this study, the pre-treated gelatin process will use pineapple waste as a solvent contains citric acid as a substitute for chemical solutions.

2. Methodology

2.1 Preparation of Pineapple Waste Liquid Extract (Atma et al., 2018)

The leftover peel and core of the pineapple obtained from the pineapple chips production in Kualu Nenas Villages, Kampar Regency, were taken to the laboratory. It is crushed using a blender to produce pulp. The pulp is then filtered and squeezed using a filter cloth to obtain pineapple waste liquid. The wastewater was then sterilized by autoclaving at 1210C, 2 atm, for 15 minutes and stored at room temperature before being used as an extracting solution.

2.2 Gelatin Extraction

Gelatin extraction was done based on the modified Atma (2018) method. Before conducting the extraction, the fish bones are first cleaned from the remnants of attached meat, boiled at a temperature of 1000C for approximately 15 minutes (Samsudin, 2018), dried, and the bone surface area is reduced. In the demineralization stage, the mashed catfish bones were soaked in 0.5 M EDTA solution (pH 7.5) for 24 hours (Theng, C.H., 2018), then washed with water. Subsequently, the catfish bones were soaked in pineapple waste liquid with a ratio of 1:5 (m/v) for 24 hours (pre-treatment stage). The bones were washed with water until the pH was neutral. The fish bone obtained is called ossein.

In the gelatin extraction stage, ossein was immersed in water with a ratio of 1:5 (m/v) at 750C for 5 hours (extraction stage). The primary extraction was done in each treatment with a mixture of fishbone ossein and water and then separated by filter paper. The liquid filtrate gained was collected in an Erlenmeyer flask. The liquid filtrate obtained is called the gelatin liquid extract. The liquid gelatin was then concentrated in a water bath at 550C and was dried at 550C in the oven. Dried gelatin, powdered.

2.3 Rendemen (AOAC, 1995)

The yield was attained from the comparison between the weight of dry gelatin.

2.4 Analysis of Physicochemical Characteristics

a. Organoleptic Examination

The organoleptic examination includes observing the shape, color, smell, and taste of the gelatin produced.

b. Gelatin pH (GMIA, 2019)

Gelatin powder is required as much as 7.5 ± 0.01 grams into a beaker and added 105 ± 0.2 grams of distilled water, then stirred until all the gelatin particles were suspended. Leave it for 1-3 hours at room temperature. Place the sample on a water bath at 650C for 10-15 minutes while stirring. Transfer the sample to a water bath with a temperature of 600C and let it remain. Calibrate the pH meter using pH 4 and pH 7 buffers at a temperature of 600C. Determine the gelatin pH liquid in accordance with the pH meter instructions. Rotate the liquid well using the pH probe to ensure the electrode is sufficiently saturated.

c. Proximate Analysis

The proximate analysis was conducted along with the procedures established by GMIA, 2019, and SNI 01-2891-1992. The proximate analysis includes water and ash content using GMIA, 2019, while protein and crude fat content using SNI-01-2891-1992.

3. Result and Discussion

The results of the study from gelatin of catfish bones with pineapple peel waste extract are:

3.1 Result

3.1.1 Rendemen

The yield resulting from this research is 2.55%.

Organoleptic

The organoleptic test results can be seen in table 1.

Table 1. Catfish Bone Gelatin Organoleptic Test Results

	Results	Standard (Farmakope V, Ditjen POM, 2012)
Form	Film sheet, coarse powder	Sheets, chips, moderately coarse powder
Color	Yellowish-brown	Weak yellow- light brown
Smells	Slight fishy smell	Weak broth

3.1.2 Physicochemical Properties

Table 2. Results of Physicochemical Properties of Catfish Bone Gelatin

	Results	GMIA (2012)	BSN (1995)
pH	4,58	3,8-5,5	4,5 -6,5
Water content (%)	11,66	< 10,5	<16
Ash content (%)	17,71	0,3-2	3,23
Crude Fat Content (%)	0,17	<5	Max 5
Protein Level (%)	64,71	>90	>87,65

3.2 Discussion

The gelatin formation from catfish bones with pineapple peel waste undergoes several stages, including demineralization, pre-treatment, and extraction—the first thing to do before the gelatin production process is making liquid pineapple waste. Pineapples are gained from Kualu Nenas, and the selected pineapples have the same maturity level. The peel and heads are taken, then cleaned from dirt and dust. After cleaning from impurities, the pineapple skin and hump are roughly chopped, then blended to minimize the surface, so the water is easily squeezed out. From 30 pineapples taken from the hump and skin, 12.5 liters of liquid pineapple waste were gained. This liquid pineapple waste was then sterilized in an autoclave at 1050C, with a pressure of 1 atm for 15 minutes. Next, it was allowed to stand at room temperature before the liquid was used as a soaking material in the pre-treatment stage.

Before the gelatin is extracted from the catfish bones, it is necessary to clean the bones from the remaining fat and meat attached, known as the degreasing process. From 30 kilograms of catfish bones from Kualu Nenas village, it was cleaned by boiling the bones at a temperature of 80-1000C for approximately 15 minutes. As claimed by Nurilmala (2017), the optimum temperature for removing fat from bone is 800C due to the melting point temperature of fat and the coagulation temperature of bone albumin, which is between 32-800C, resulting in optimum fat solubility. If the temperature exceeds this limit, it is feared that the fat in the extracted bone will run out, then boiling will decompose the protein contained, and the protein will be damaged first due to boiling. It can cause the percentage yield of gelatin to be small.

Furthermore, demineralization is done where there is a process of removing minerals in the bone so that ossein is acquired. In this process, the catfish bones were soaked in 0.5 M EDTA solution (pH 7.5) for 24 hours to diminish the bones' ash content. This process intensely determines the ash content in catfish bone gelatin (Theng, C.H., 2018). After soaking with EDTA solution, the bones were cleaned with distilled water, then soaked with liquid pineapple waste with a 24-hour soaking treatment. All of these soaking processes are done to remove calcium and mineral salts from the bones to become soft or called ossein contains collagen.

During the process, the catfish bones are stirred occasionally. In gelatin manufacture, the treatment of animal collagen raw materials with dilute acid or base affects the cross-linking of proteins to be cut, the structure to be broken, and the pieces to be dissolved in water. These water-soluble pieces of protein chains are called gelatin. The quality of gelatin produced depends on the concentration of acid or base used, temperature, and length of immersion time (GMIA, 2012).

In this study, pineapple peel waste liquid was used as an ingredient for soaking acidic fish bones; it is a natural source comprises high citric acid (Hajar et al., 2012). Citric acid is an organic acid has the most role in the acidity level of pineapple waste. The content of citric acid in pineapple waste is about 2.18 g per liter (Abdullah & Mat, 2008). Citric acid as a solvent in the pre-treatment stage of gelatin extraction is better than other organic acids (Mariod & Adam, 2013). After soaking with pineapple solution, the solution is removed and washed until the pH is neutral; the bone resulting from this washing is called ossein.

In the foremost extraction step, ossein was extracted with distilled water in a ratio of 1:5 (m/v) at 750C for 5 hours. Based on research by Atma (2017), which identified gelatin extracted from catfish bones using pineapple peel were using two extraction temperature conditions, namely 650C and 750C, it was found that at 750C extraction temperature showed high gelatin content, in line with (Pertwi's research, 2018) which extracted fish bone using citric acid with various extraction temperatures of 45, 55, 65, and 750C for 5 hours, gained the best fishbone gelatin with a temperature of 750C for 48 hours of immersion. While the central extraction time of 5 hours is the optimum time because if it is more than 5 hours, the ossein will be destroyed and dissolved with distilled water (Rahayu & Fithriyah, 2015). The gelatin liquid was filtered using filter paper. It was concentrated in a water bath at 550C. The concentrated liquid was put into an oven at 550C to dry on gelatin sheets, then powdered.

3.3 The Yield

Yield is a imperative parameter in the gelatin isolation process. The greater the yield produced, the more influential the resulting treatment. The yield produced in this research is 2.55. These results indicate that the longer the immersion, the higher the yield. As said by Park (2013), the longer the immersion in acid, the weaker the bonds in the collagen, which causes more significant swelling, and more gelatin is produced. The yield value produced is still low. The acid used in the demineralization process of catfish bone may be weak. The activity of the acid solution is not optimal in loosening the collagen chain bonds and cross-linking between the collagen (Ramdani, 2014).

In line with the statement of Courts and Johns (1977) that the process of bone demineralization into ossein affects the success rate (yield) of collagen extraction in ossein. This collagen will then be converted into gelatin. Furthermore, the concentration factor of the acid solution and the immersion time are essential factors in the demineralization process. In a study conducted by Jaya (2020), who extracted snakehead fish bone gelatin with a variety of acids, namely synthetic citric acid, starfruit acid, and lime acid, the average yield values were 6%, 2.32%, and 3.33%, where Synthetic citric acid has a higher yield value than the yield value of organic acids. It is in line with the yield value of the pineapple peel waste used.

When compared with the study results of extracting gelatin from tilapia bones with several immersion times in 5% HCl, the yield percentage was between 7.34 to 10.20%. It presents the yield percentage is not too far from the yield of the research results.

3.4 Acidity (pH)

The pH value is the acidity level of the gelatin, which is vital for analysis. Gelatin pH affects other gelatin properties and also affects the application in the product. The pH value of gelatin is related to the process used. Acid processes tend to produce low pH values, while alkaline tends to produce high pH values. Gelatin with a neutral pH tends to be preferred, so the neutralization process has a vital role in neutralizing the remaining acid after soaking.

The pH value in this study was 4.58. It still meets the gelatin standard required by GMIA (2012); acid-made gelatin has a value range of 3.8-5.5, and the standard required by BSN (1995) is 4.5-6.5. In line with previous research, in catfish bone gelatin (*Pangasius sutchi*) with pineapple waste immersion, the pH value was 4.52 (Atma, 2018), snakehead fish bone gelatin with citric acid, lime acid, and starfruit acid immersion, respectively. i.e., 6.13; 6.80; 6.83 (Jaya, 2020), and the pH of catfish bone gelatin with citric acid immersion is 4.462 (Pertiwi, 2018).

3.5 Water content

Water content is one of the crucial tests in determining the quality of gelatin. It will affect the shelf life of gelatin because it is closely related to metabolic activities that occur while the gelatin is stored, such as enzyme activity, microbial activity, chemical activity, namely the occurrence of rancidity and non-enzymatic reactions, resulting in changes in gelatin properties quality and the value of gelatin quality (Darwin, 2018).

In this study, the value of the water content was 11.66%. The results of this study are included in the requirements set by SNI 06-3735-1995 (BSN, 1995) and the European Pharmacopoeia (European Pharmacopoeias Commission, 2014), which are a maximum of 16% and 15% but have not yet entered the requirements determined by the GMIA (2012) which should not exceed 10.5%. The value of this concentration is influenced by the amount of collagen formed in gelatin, which causes hydrogen bonds from non-collagen to bind to water molecules so the drying process will evaporate along with water. The longer the immersion, the lower the water content produced. The more open collagen structure causes the binding capacity of gelatin to adsorb water to be more significant, and the binding capacity to free water is low. The weaker binding capacity to free water causes the water in gelatin to evaporate quickly during drying and produces low water content (Ulfah, 2011).

Previous research on the extraction of catfish bone gelatin (*Pangasius sutchi*) by soaking pineapple waste is 8.59% (Atma, 2018). Research on extracting snakehead fish bone gelatin by soaking citric acid, lime acid, and wuluh starfruit acid, respectively. 9.21%, 11.29%, and 18.96%, while the catfish bone gelatin with citric acid immersion was 7.72%.

3.6 Ash Level

Measurement of ash content aimed to determine the mineral content in gelatin. Ash content affects gel strength and protein content. The purer the gelatin, the higher the protein content. The high ash content is due to calcium, potassium, sodium, iron, and magnesium in bones (Yenti, 2016). The ash content produced in this study was 17.71%. This value exceeds the requirements set by GMIA (2012), which is 0.3-2%, and BSN (1995), which is 3.25%.

Ash content exceeds the standard indicates the gelatin is not pure. Factors affecting the high ash content are the filtering process, raw materials content, and the extraction process (Du, 2013). Meanwhile, concerning Panjaitan (2016), the high ash content of fish bone gelatin may be caused by the minerals are still bound to collagen due to an imperfect demineralization process.

3.7 Crude Fat Content

Determination of fat content affects the material quality during storage. High-quality gelatin has a low-fat content (Shyni, 2014). In this study, the fat content value was 0.17%. It meets the standard by GMIA (2012) less than 5% and BSN (1995), a maximum of 5%. As attested by Winarno (2004), in general, the use of acids can accelerate hydrolyzing fats into glycerol and fatty acids. The higher the acid, the more the fat will decompose, thereby decreasing the fat content of the gelatin produced. The fat content value

of this study was lower than that of catfish bone gelatin with citric acid immersion, which was 2.79% (Pertiwi, 2018). Likewise, the fat content of catfish bone gelatin (*Pangasius sutchi*) by soaking pineapple skin waste is 7.71%.

3.8 Protein Level

Gelatin is a type of conversion protein produced through the hydrolysis of collagen. The test shows how much protein content is in a food ingredient (Ulfah, 2011). The results of this study revealed that the protein content is 64.71%. This value is still far from the requirements determined by GMIA (2012), protein content of more than 90% and conforming to SNI (1995) of 87.65%. The low protein content results in the gelatin sample can be caused by several factors, including the high extraction temperature, which can cause further hydrolysis, so that the protein in the gelatin sample can be broken down into more superficial elements such as its constituent elements, namely C, N, O., and H (Yenti, 2015).

From a previous study conducted by Pertiwi (2018), catfish bone gelatin with citric acid immersion, the protein content was 57.80%, and research on mackerel bone gelatin by soaking in some acid solutions obtained a protein content of 58.37%. Atma's (2018) research, namely the extraction of catfish bone gelatin (*Pangasius sutchi*) by soaking pineapple skin waste, obtained a protein content of 47.60%.

4. Conclusion

Catfish bone gelatin (*Pangasius hypopthalmus*) extracted by soaking pineapple peel waste has physicochemical properties, among others; yield 2.55, pH 4.58, water content 11.66%, ash content 17.71%, crude fat content 0.17%, protein content 64.71%, some physicochemical characteristics of gelatin in this study meets the standard of gelatin quality. The Promethee-based decision support framework assists decision-makers in determining the performance of lecturers based on the guidelines presented.

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