World Journal of Pharmaceutical and Life Sciences
WJPLS
www.wjpls.org
SJIF Impact Factor: 6.129

# IDENTIFICATION THE CHEMICAL PROPERTIES OF CATFISH BONE WASTE (PANGASIUS HYPOPHTALMUS) GELATIN BY UTILIZING AN ORGANIC LIQUID OF PINEAPPLE PEEL WASTE (ANANAS COMOSUS)

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**Article Received on 14/07/2023** **Article Revised on 03/08/2023** **Article Accepted on 24/08/2023**

## 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 identification the chemical properties qualitatively 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 6, 12 and 24 hours. While in the central extraction, ossein will be immersed in water at a temperature of $75^0$C for 5 hours. The gelatin chemical testing qualitatively results for bond peptide of the three treatments of immersion time formed a puprple color ; for Triptophan content of the three treatment of immersion time showed that does not contained the amino acid of tryptophan; and for Sulfur test that showed were does not formed of black to brown precipitate. This showed that catfish bone gelatin that can be seen from the amino acid composition formed.

**KEYWORDS:** Identification, Gelatin, Catfish Bone.

## INTRODUCTION

The demand for gelatin is intensifying every year; gelatin from pork is the primary source on the market. In 2007, 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%.[1] Only about 1% comes from other sources.[2]

Kampar Regency, Riau Province, is one of the centers of catfish cultivation in Indonesia. Catfish processed into fillets have a higher economic value.[3] This processing produces waste of fish skin and bones. It causes problems for the environment since its utilization has not been maximized.[4] 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[5] reveals that catfish bones produce gelatin with a higher concentration than gelatin from other fish bones. It is in line with research from[6] which mentions gelatin from catfish (Pangasius hyphothalmus) products produce gelatin compared with commercial gelatin.[7] 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.[8;9]

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.[10] 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%.[11]

On this occasion, the researchers would like to research the physicochemical properties of gelatin derived from the catfish bones (Pangasius hypopthalmus) 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.

## MATERIALS AND METHODS

*Preparation of Pineapple Waste Liquid Extract*[12]

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 $121^0$C, 2 atm, for 15 minutes and stored at room temperature before being used as an extracting solution.

*Gelatin Extraction*

Gelatin extraction was done based on the modified Atma, 2018[12] method. Before conducting the extraction, the fish bones are first cleaned from the remnants of attached meat, boiled at a temperature of $100^0$C 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 6 hours, 12 hours and 24 hours[13], 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 $75^0$C 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 $55^0$C and was dried at $55^0$C in the oven. Dried gelatin, powdered.

*Gelatin Chemical Testing Qualitatively*

a. Bond Peptide

This test is carried out with Biuret test method. Biuret's solution consist of on CuSO4 and KNa-tartate in NaOH. 3 ml 2% gelatin solution 1 ml of 10% NaOH wa added and shaken. After that added 1mL of Biuret reagent. Then shaken back and heated. Reaction positive if it produces a purple color.

b. Triptophan Content

This test is carried out with Hopkins-Cole test method. Gelatin solution 2% as much as 1 mL added acid of 5% glyoxylate and shaken. Then add concentrated $H_2SO_4$ as much as 1 mL. Positive reaction when produce a purple ring.

c. Sulfur Analysis

Testing is carried out by testing sulphur precipitation. Element sulphur (S) can be found in two amino acids, namely cysteine and methionine. 1 ml of 2% gelatin solution was added 1 ml of 10% NaOH and heated. After that 1 drop of 5% Pb acetate was added. Positive reaction when it turns yellow then it becomes brown and finally settles black.

## RESULTS AND DISCUSSION

### RESULT

The results of the gelatin qualitative test analysis from each treatment can be seen in the following table.

**Table 1: Qualitative Test Result.**

| Test | Treatment | Result | Pharmakope V |
|---|---|---|---|
| **Biuret Test (Peptyde Bound)** | P1 | Purple | Purple |
| | P2 | Purple | |
| | P3 | Purple | |
| **Hopkins-Cole Test (Triptofan)** | P1 | No purple ring formed | Purple Ring Formed |
| | P2 | No purple ring formed | |
| | P3 | No purple ring formed | |
| **Sulphur** | P1 | No precipitate is formed | Yellow, then brown and finally black |
| | P2 | No precipitate is formed | |
| | P3 | No precipitate is formed | |

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 $105^0$C, 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-$100^0$C for approximately 15 minutes. As claimed by Nurilmala, 2017[14], the optimum temperature for removing fat from bone is $80^0$C due to the melting point temperature of fat and the coagulation temperature of bone albumin, which is between 32-$80^0$C, 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 (13). After soaking with EDTA solution, the bones were cleaned with distilled water, then soaked with liquid pineapple waste with the 6 hour, 12 hour and 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.[15] 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.[11] 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.[16] Citric acid as a solvent in the pre-treatment stage of gelatin extraction is better than other organic acids.[17] 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 $75^0$C for 5 hours. Based on research by Atma.[12], which identified gelatin extracted from catfish bones using pineapple peel were using two extraction temperature conditions, namely $65^0$C and $75^0$C, it was found that at $75^0$C extraction temperature showed high gelatin content, in line with[18] which extracted fish bone using citric acid with various extraction temperatures of 45, 55, 65, and $75^0$C 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.[19] The gelatin liquid was filtered using filter paper. It was concentrated in a water bath at $55^0$C. The concentrated liquid was put into an oven at $55^0$C to dry on gelatin sheets, then powdered.

**Peptide Bond Test (Biuret Test)**

Gelatin is composed of amino acids that form peptide bonds. This test is used to determine the presence of peptide bonds in gelatin extracted from catfish bones. The results of the biuret test will form a purple color which indicates the presence of a peptide bond, where the longer the peptide bond, the purple color formed will be clearer and darker. The results of this study indicate that catfish bone gelatin has peptide bonds, where the results of the three treatments of immersion time form a purple color.

**Hopkins-Cole Test**

The Hopkins-Cole test is specific for proteins containing the amino acid tryptophan. To determine the presence of tryptophan in protein through the Hopkins-Cole test it will form a purple ring. The result of this study indicate that the gelatin extracted from catfish bones does not contain the amino acid tryptophan because it does not form a purple ring, this is in accordance with the literature, where the amino acid composition of both gelatin type A and type B does not contain the amino acid tryptophan.

**Sulfur Analysis**

Element sulfur (S) can be found in two amino acids, namely cysteine and methionine. According to the literature, gelatin does not contain the amino acid cysteine. Based on the test results in this study, it was not found that none of gelatin produced contained the amino acid cysteine, this was seen from the results, where the Pb-acetate reaction with the amino acid would form a brownish to black precipitate. However, from this test the reaction did not occur marked by the absence of the formation of the black to brownish precipitate.

## CONCLUSION

Catfish bone gelatin (Pangasius hypophtalmus) extracted by soaking pineapple peel waste has qualitative test, among peptide bond test, Hopkins-Cole test and Sulfur analysis showed that were in accordanve with the conditions for the presence of amino acids in gelatin.

## ACKNOWLEDGEMENTS

Thank you to the Poltekkes Kemenkes Riau for financial support and the smooth running of the research, as well as to all parties involved.

## REFERENCES

1. Gómez-Guillén, M.C., Pérez-Mateos, M., Gómez-Estaca, J., López-Caballero, E., Giménez, B. and Montero, P. (2009). Fish gelatin: a renewable material for developing active biodegradable films. Trends in Food Science & Technology, 20(1): pp.3-16.
2. Ahmad, M. and Benjakul, S. (2011). Characteristics of gelatin from the skin of unicorn leatherjacket (Aluterus Monoceros) as influenced by acid pre-

treatment and extraction time. Food Hydrocolloids, 25(3): pp.381-388.
3. Kemendag RI. (2013). Ikan patin hasil alam bernilai ekonomi dan berpotensi ekspor tinggi Warta ekspor, Ditjen PEN/MJL/004/10/2013: 3-11.
4. Agustin, A.T. (2013). Gelatin ikan: sumber, komposisi kimia dan potensi pemanfaatannya. Media Teknologi Hasil Perikanan, 1(2).
5. Mahmoodani, F., Ardekani, V.S., Fern, S.S., Yusop, S.M. and Babji, A.S. (2014). Optimization of extraction and physicochemical properties of gelatin from pangasius catfish (Pangasius sutchi) skin. Sains Malaysiana, 43(7): pp.995-1002.
6. Oktaviani, I., Perdana, F. and Nasution, A.Y. (2017). Perbandingan sifat gelatin yang berasal dari kulit ikan patin (Pangasius hypophthalmus) dan gelatin yang berasal dari kulit ikan komersil. JOPS (Journal Of Pharmacy and Science), 1(1): pp.1-8.
7. Zhang, F., Xu, S., & Wang, Z. (2011). Pre-treatment Optimization and Properties of Gelatin from Freshwater Fish Scales. Food and Bioproducts Processing, 89(3): 185-193.
8. Badii, F. and Howell, N.K. (2006). Fish gelatin: structure, gelling properties and interaction with egg albumen proteins. Food hydrocolloids, 20(5): pp.630-640.
9. Ratnasari, I., Yuwono, S.S., Nusyam, H. and Widjanarko, S.B. (2013). Extraction and characterization of gelatin from different freshwater fishes as alternative sources of gelatin. International Food Research Journal, 20(6): p.3085.
10. Nur, S., Surati, S. and Rehalat, R. (2017). Aktifitas enzim bromelin terhadap peningkatan protein tepung ampas kelapa. Biosel: Biology Science and Education, 6(1): pp.84-93.
11. Hajar, N., Zainal, S., Nadzirah, K.Z., Roha, A.S., Atikah, O. and Elida, T.T. (2012). Physicochemical properties analysis of three indexes pineapple (Ananas Comosus) peel extract variety N36. APCBEE Procedia, 4: pp.115-121.
12. Atma, Y., Ramdhani, H., Mustopa, A.Z., Pertiwi, M. and Maisarah, R. (2018). Physical-chemicals Characteristic of Fish Bone Gelatin from Pangasius catfish Extracted Using Pineapple Waste. AGRITECH-JURNAL TEKNOLOGI PERTANIAN, 38(1): pp.56-63.
13. Theng, C.H., N. Huda, N.A.N. Muhammad, C. Wariyah, H. Hashim. (2018). Physicochemical Properties of Duck Feet Collagen with Different Soaking Time and Its Application in Surimi. International Journal on Advanced Science Engineering Information Technology, 8(3): 832-841.
14. Nurilmala, M., Jacoeb, A.M. and Dzaky, R.A. (2017). Quality of cultured wader pari during storage at different temperature. Jurnal Pengolahan Hasil Perikanan Indonesia, 20(2): pp.339-350.
15. GMIA. (2012). Gelatin Handbook. USA: Gelatin Manufacturers Institute of America.
16. Abdullah & Mat, H. (2017). The Characteristic of Pineapple Waste from Canning Industry. Advanced Science Letters, 23(6): 5691-5693.
17. Mariod, A.A. and Fadul, H. (2013). Gelatin, source, extraction and industrial applications. Acta Scientiarum Polonorum Technologia Alimentaria, 12(2): pp.135-147.
18. Pertiwi, M., Y. Atma, A. Z. Mustopa, R. Maisarah. (2018). Karakteristik Fisik dan Kimia Gelatin dari Tulang Ikan Patin dengan Pre-Treatment Asam Sitrat. Jurnal Aplikasi Teknologi Pangan, 7(2): 83-91 Pertiwi, M., Atma, Y., Mustopa, A.Z. and Maisarah, R., 2018. Karakteristik fisik dan kimia gelatin dari tulang ikan patin dengan pre-treatment asam sitrat. Jurnal Aplikasi Teknologi Pangan, 7(2).
19. Rahayu, F., Fithriyah, N.H. (2015). Waktu Ekstraksi terhadap Rendemen Gelatin dari Tulang Ikan Nila Merah. Prosiding Seminar Nasional Sains dan Teknologi, Jakarta: 17 November.