Studii şi Cercetări Ştiinţifice 2020, **21** (4), pp. 547 – 553
Chimie şi Inginerie Chimică, Biotehnologii, Industrie Alimentară

Scientific Study & Research
Chemistry & Chemical Engineering, Biotechnology, Food Industry *ISSN 1582-540X*

ORIGINAL RESEARCH PAPER

ISOLATION AND CHARACTERIZATION OF COLLAGEN FROM SNAKEHEAD (*CHANNA STRIATA*) SKIN WITH DIFFERENT SOLVENTS AND SOAKING TIME

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Received: October, 01, 2019
Accepted: November, 26, 2020

Abstract: The objective of this research was to utilize snakehead skin waste as an alternative source of collagen. Different solvents (acetic acid, chloride acid and citric acid) and different soaking time (three days and five days) were employed as treatments in this study. Parameters observed were yield, protein solubility, and molecular weight. The results showed that acetic acid is the optimal solvent for extracting the snakehead skin collagen as indicated by the highest yield with an average value of 84.07 %. The collagen with highest protein solubility was produced by soaking the snakehead skin in acetic acid for 5 days. The results of SDS-PAGE analysis revealed 14 kinds of protein in a collagen. Each different solvent produced collagen with various molecular weights which ranged from 12.39 to 185.70 kDa.

Keywords: *acetic acids, fish collagen, soaking, solvent, snakehead*

INTRODUCTION

Collagen is the main protein that makes up about 30 % of the total protein in the animal body. Collagen presents in many tissues such as the skin and bones. Collagen is widely used in foodstuff, pharmaceuticals, cosmetics, biomedical materials and leather industry. The main source of the collagen in the industry comes from the skin and bones of cows and pigs. The outbreak of mad cow disease has led cow collagen users to search for an alternative. Moreover, the collagen from skin and pig bones can't be used in some areas for religious reasons. Waste from fish such as bones and scales as well as skin contains collagen [1] and they are very potential to be used as industrial collagen source.

Research on the use of aquatic organisms as a source of collagen has been increasing [2 – 7]. Collagen content of carp (*Cyprinus carpio*) skin is 41.30 %, 1.35 % in fish scales and 1.06 % in fish bone [8]. To the best of our concern, the isolation and characterization of collagen from the skin of snakehead fish (*Channa striata*) with different acid types as solvent and different soaking time has not been done. Therefore, this research is important to initiate the use of snakehead fish waste which is abundant in some regions in Indonesia as a collagen source.

MATERIALS AND METHOD

The materials used in this study were acetic acid, HCl, NaOH, acrylamide, bisacrylamide, TEMED (N,N,N,N-tetramethylethylendiamin), ammonium persulfate, *β*-mercaptoethanol, sodium dodecyl sulfate (SDS), glacial acetic acid, bromphenol blue, and glycine and were procured from Merck, Germany, and Coomassie Brilliant Blue R-250 from Sigma-Aldrich, Germany. The tools used include OHAUS analytical balance (USA), micropipette (single channel capp 10-100 Ul, USA), hotplate (Cimarec, United Kingdom), cold centrifugation (model MRX-152), spectrophotometer (Phramacia LKB-Novaspec II, Netherlands) and incubator (model Certomat, Sartorius Group, Germany), SDS-PAGE (Bio-Rad, USA) electrophoresis equipment.

Collagen extraction

Collagen was extracted from snakehead fish (*Channa striata*) skin following Baehaki method [9]. To remove non-collagenous proteins, the skin was mixed with 0.1 mol·L^{-1} NaOH in a ratio of 1 : 10 (w/v), followed by continuous stirring for 6 h using an overhead stirrer. The alkali solution was changed every 2 h. Pretreated fish skin was soaked in 1.5 % acetic acid with a solid to solvent ratio of 1 : 2 (w/v) for 24 h. Skin was washed with cold water until neutral *p*H, followed by extraction with distilled water with a solid to solvent ratio of 2 : 1 (w/v) for 3 h at 50 ℃.

Extraction yield

The yield of collagen is determined based on the ratio between the wet weight of collagen produced and the weight of the fish skin sample used.

$$\text{The Yield} = \frac{\text{The wet weight of collagen}}{\text{The Weight of the fish skin sample used}} \text{ x } 100\ \% \quad (1)$$

Solubility of proteins

A total of 0.5 g of the sample was dispersed in 20 mL of water, then the *p*H was adjusted to 2, 4, 6, 8, 10, 12 with 0.1 NaOH and 0.1 N HCl. The sample was then stirred with a 250 rpm stirrer for 30 min at room temperature (25 ℃), after which the sample was centrifuged (4,000 rpm) for 10 min. Supernatant of centrifuge result is measured for dissolved nitrogen by Bradford method [10].

$$\text{Solubility of protein} = \frac{\text{Nitrogen in supernatan (\%)}}{\text{Nitrogen total (\%)}} \text{ x } 100\ \% \quad (2)$$

Determination of molecular weight

Molecular weight was estimated by electrophoresis under denaturating polyacrylamide-SDS (SDS-PAGE) with 8 % polyacrylamide gels [11]. The standard molecular weight markers were phosphorylaseb (97 kDa), bovine serum albumin (66 kDa), ovalbumin (45 kDa), carbonic anhydrase (30 kDa) and lysozyme (14.3 kDa).

RESULT AND DISCUSSION

Yield

The resulting yield was calculated based on the comparison between the collagen produced and the weight of the fish skin used. The yield calculation is done to determine the percentage of collagen produced since it is directly proportional to the effectiveness and efficiency of the treatment used. The final collagen form of snakehead (*Channa striata*) skin in this study was wet collagen, without further drying process. The average yield ranged from 3.25 to 43.43 %, as presented in Figure 1.

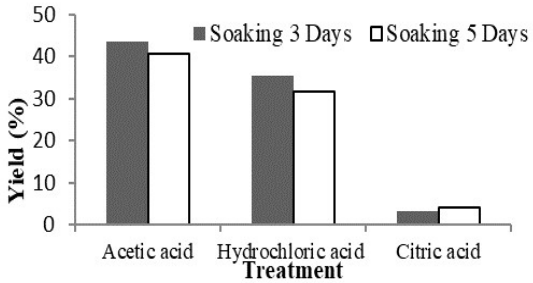


Figure 1. *Collagen yield of Snakehead (Channa striata) skin with acid solvent treatment and different soaking time*

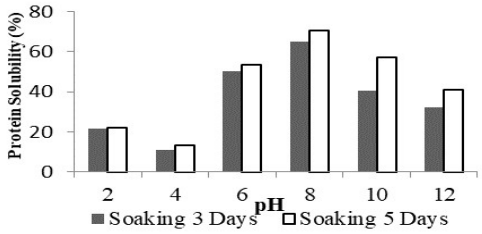
Figure 1 shows that the highest yield was produced in snakehead skin soaked in acetic acid solvent for 3 days while the lowest was in the treatment of citric acid solvent with 3 days soaking time. Collagen yield with acetic acid solvent treatment in this study was higher than that of collagen yield from research of Wulandari *et al* [12]. As a

comparison, the extraction yield of collagen from bighead carp was 17.50 % [13] and from striped catfish was 7.70 % [14].

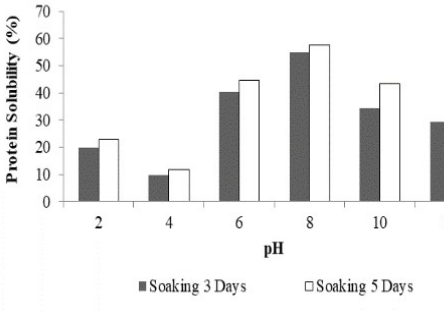
The use of acetic acid as solvent in the process of protein extraction leads to protein denaturation. This is due to the ability of partially ionized acetic acid to enter the cell of the skin and denature the triple helix bond into a single helix and produce greater yield. According to Yuan [15], acetic acid has the ability to swell (swelling capacity) which is able to weaken the bonds between collagen molecules so as to increase the results of extraction. Extraction yield of collagen with HCl solvent was lower than that of acetic acid. The decrease in yield was due to the nature of hydrochloric acid as a strong acid which results in the presence of excess H^+ ions. The electronegativity of strong acids is greater so that the electron bonds are stronger than the hydrogen atoms. The strength of acid increases with the increase in electronegativity of X atoms on H-X. As the most abundant amino acids in collagen are glycine, proline and hydroxyl-proline, the addition of strong acid like HCl does not provide optimum yield. The use of 5 % citric acid solvent has the lowest yield. This is presumably due to the low electronegativity properties of citric acid. The citric acid is a weak acid with low coagulation power that prevents extraction from running optimally. In the study of Skierka and Sadowska [16] which isolated collagen from cod skin with the use of 0.5 M citric acid and three days soaking time produced a low yield which ranged from 0- 17 %. This shows that the coagulation power and electronegative properties of citric acid solvents affect the collagen extraction yield.

Protein Solubility

Protein solubility is the amount of nitrogen in protein dissolved in water under certain conditions. Protein solubility is a physicochemical property associated with other functional properties. Understanding the protein solubility would provide useful information in the utilization of the functional properties of proteins. The effect of acid solvents on collagen protein solubility is shown in Figure 2.



A



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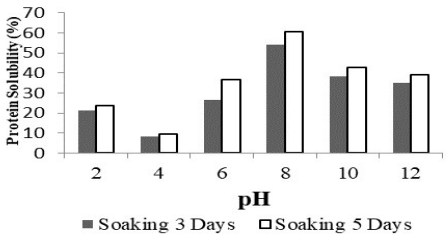

C

Figure 2. *Effect of acid solvents on the solubility of collagen protein*
(A = acetic acid, B = hydrochloric acid and C = citric acid)

The highest collagen solubility (70.42 %) was found in the sample produced by soaking in acetic acid solvents for 5 days, while the lowest was the collagen extracted after treatment with citric acid for 3 days. All collagen produced with various acid types used in this research showed the same tendency to increase in their solubility above *p*H 4 and reach the optimum at *p*H 8.

Molecular Weight

Molecular weight determination is performed using SDS-PAGE which results are shown in Figure 3.

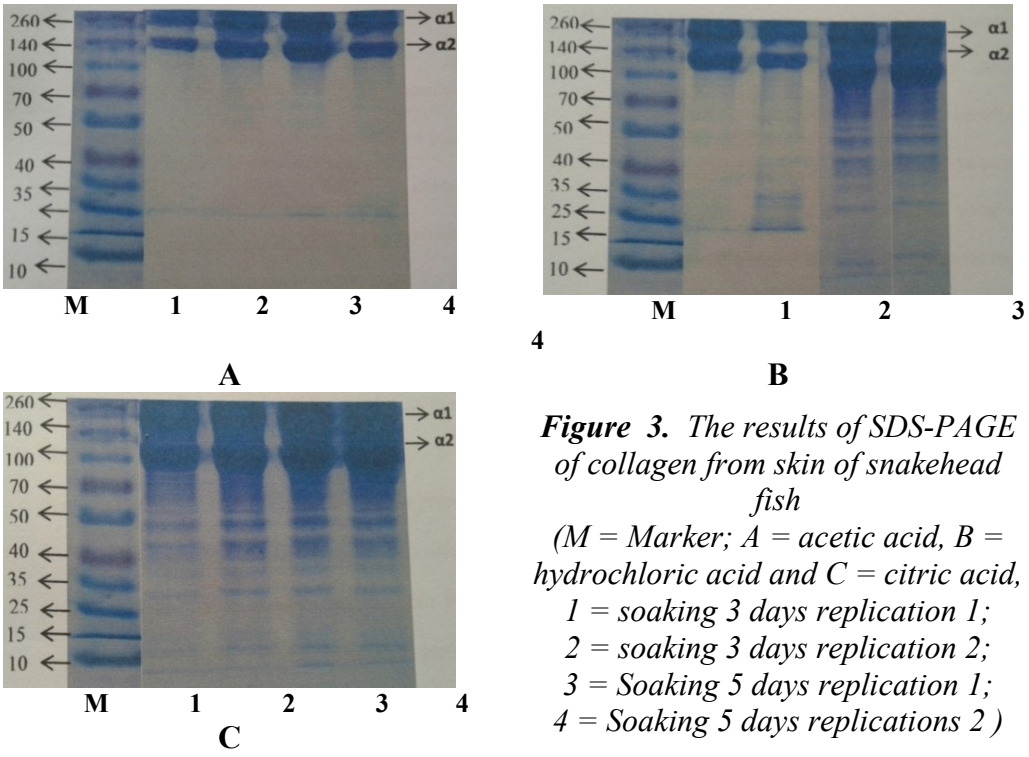

Figure 3. *The results of SDS-PAGE of collagen from skin of snakehead fish (M = Marker; A = acetic acid, B = hydrochloric acid and C = citric acid, 1 = soaking 3 days replication 1; 2 = soaking 3 days replication 2; 3 = Soaking 5 days replication 1; 4 = Soaking 5 days replications 2)*

Based on the results of SDS-PAGE each ribbon that appears and disappears leaves the migration distance pattern almost the same but the number of bands that appear were different. Collagen which was extracted after soaking in acetic acid for 3 days had 2 bands, while in 5 days sample were 3 bands. The collagen produced by soaking in chloride acid solvent for 3 and 5 days obtained 3 bands and 18 bands respectively. Lastly, the use of citric acid solvents to soak the snakehead skin for both 3 and 5 days produced collagen with 18 bands.

The results of SDS-PAGE analysis showed that the longer the soaking time, the more ribbons obtained. It is an indication that the collagen extraction results were not optimal. The difference between the solvents used showed that the acetic acid solvent obtained 2 bands which showed both bands were collagen, whereas the use of solvents of hydrochloric acid and citric acid contained many bands which were the result of further hydrolysis of collagen into smaller size proteins, peptides and amino acids. According to Skierka and Sadowska [16] collagen obtained by extraction with hydrochloric acid and citric acid has a relatively small protein fragment (less than 116 kDa) due to the

degradation of collagen during the extraction process. On the other hand, collagen which was extracted with acetic acid showed high molecular weight protein. This indicated that only the α-component was extracted and no further enzymatic breakdown took place.

CONCLUSION

The acetic acid is the optimal solvent for extracting the snakehead skin collagen as it provided the highest extraction yield with an average value of 84.07 %. The highest protein solubility produced by soaking the snakehead skin in acetic acid solvent for 5 days. Collagen extracted after soaking in each different of solvents had various molecular weight which ranged from 12.39 to 185.70 kDa.

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