JIPK. Volume 15 No 2. November 2023
Sinta 1 (Decree No: 158/E/KPT/2021)
e-ISSN:2528-0759; p-ISSN:2085-5842
Available online at https://e-journal.unair.ac.id/JIPK

JIPK
(JURNAL ILMIAH PERIKANAN DAN KELAUTAN)
Scientific Journal of Fisheries and Marine

Short Communication

Effect of Acetic Acid Pre-treatment on Hydro-extraction of Water-Soluble Collagen from Skin of Alaska Pollock (*Theragra chalcogramma*)

Patmawati[1*], Aliffiansyah Rizky Ergion[2], Laksmi Sulmartiwi[1], Siva Raseetha[3,1], Dwitha Nirmala[1], Yaowapha Waiprib[4], and Sapta Wijayanti[5]

[1]Department of Marine, Faculty of Fisheries and Marine, Universitas Airlangga, Surabaya, 60113. Indonesia
[2]Undergraduate Program of Aquatic Product Technology, Faculty of Fisheries and Marine, Universitas Airlangga, Surabaya, 60113. Indonesia
[3]Faculty of Applied Sciences, Universiti Teknologi MARA, Shah Alam, Selangor, 40450. Malaysia
[4]Departement of Fishery Products, Faculty of Fisheries, Kasetsart University, Chatuchak, Bangkok, 10900. Thailand
[5]Departement of Compliance, PT Kelola Mina Laut, Gersik, East Java 61121. Indonesia

OPEN ACCESS

ARTICLE INFO

Received: December, 06 2023
Accepted: January, 20 2023
Published: February, 16 2023
Available online: Aug, 15 2023

*) Corresponding author:
E-mail: patmawati@fpk.unair.ac.id

Keywords:
Fish Skin
Fisheries
Marine Pollution
Waste Valorization
Water-Soluble Collagen

Abstract

To date, there is no information on the skin of Alaska Pollock (*Theragra chalcogramma*) being used as a source for collagen. In order to produce water-soluble collagen from the skin of Alaska Pollock, a process known as hydro-extraction is utilized. This technique does not need a long extraction time or a large amount of chemical reagent. The purpose of this study was to investigate the effect of acetic acid pre-treatment on hydro-extraction of water-soluble collagen from Alaska Pollock skin. The skin samples were pretreated using acetic acid at different concentrations (0.01 M; 0.05 M; 0.1 M, 0.15 M) for two hours at chilling temperature. The pre-treated skin samples were further processed to produce water-soluble collagen with the hydroxy-extraction method. The obtained collagen was analyzed for proximate compositions, yield, and amino acids compositions with high performance liquid chromatography. It was found that the proximate compositions of the collagen products, specifically the protein content (75%) and fat content (1%), met the requirements of the Indonesian National Standard (SNI 8076:2014). The collagen yield ranged from 2.6 to 3.13%. The predominate amino acids in collagen were glycine, arginine, proline, glutamic acid, serine, and alanine. Pre-treatment of skin sample with 0.15 M acetic acid resulted in the highest yield of water-soluble collagen (3.13%) and protein content (91.13%). The skin of Alaska Pollock fish could be used as an alternative raw material to produce water-soluble collagen for medical, pharmacy or food processing applications.

Cite this as: Patmawati, Ergion, A. R., Sulmartiwi, L., Raseetha, S., Nirmala, D., Waiprib, Y., & Wijayanti, S. (2023). Effect of Acetic Acid Pre-treatment on Hydro-extraction of Water-Soluble Collagen from Skin of Alaska Pollock (*Theragra chalcogramma*). *Jurnal Ilmiah Perikanan dan Kelautan*, 15(2):468–477. http://doi.org/10.20473/jipk.v15i2.41274

1. Introduction

The fishery product processing industry is a business sector that has promising sustainability due to the abundant availability of fishery resources, human resources, and a wide range of market opportunities (KKP, 2013). However, the fishery processing industry also often produces abundant amount of waste in each production stage. The fishery waste consists of liquid waste and solid waste. Liquid waste can be in the form of blood, mucus, and fat, while solid waste consists of heads, fins, skin, bones, and scales. Fish skin is one of the by-products that has not been used optimally so far and there is low effort in processing fish skin waste, thus, resulting in waste problem that contaminate and damage environmental aesthetics. Currently, the fish skin byproducts from fish processing has limited uses as a mixture of feed, handicrafts, processed fish skin crackers and so on. Therefore, another alternative is needed to handle fish skin waste as solution to reduce environmental impact and become a product that has added value, namely as a raw material for making collagen.

Collagen is an extracellular matrix (ECM) glycoprotein that is affected by the raw material used (Chen *et al*., 2022). Collagen is a protein with a fibrous structure which is the main component of the extracellular matrix of a living organism, which accounts for 25-30% of the total protein and plays an important role in maintaining the integrity of the biological structure of several tissues (Maroušek *et al*., 2015; Jafari *et al*., 2020). Collagen is the main structural component of white connective tissue fibers and is present in tissues and organs that play an important role in the preparation of body shape (Walters and Stegemann, 2014). Collagen is widely used in various field including industry, food, cosmetics, pharmaceuticals, medicine, photography and so on. Most of the commercial collagen in circulation comes from the tissues and skin of terrestrial animals such as pigs, cow, or chickens (Silvipriya et al., 2015). It is feared that the use of collagen can cause diseases such as mad cow (Mad Cow), Bovine Spongiform Encephalopathy (BSE), Transmissible Spongiform Encephalopathy (TSE), Foot and Mouth Disease (FMD) which can be transmitted to humans resulting on a negative impact on human health. Collagen from cows and pigs also raises other problems when viewed from a religious perspective (Singh *et al*., 2011). Kusumawinahyu *et al*. (2022) reported that collagen from Pangasius skin with immersing using acetic acid for 72 hours resulted high yield (6.15%) and protein content (9.26%).

Fish is one of the fisheries commodities that can be used as an alternative raw material for collagen which is not limited by religious barrier, safe and has a good impact on health. Collagen from fish and collagen from land animals differs from their high biological value, high content of essential amino acids, and low content of hydroxyproline (Muralidharan *et al*., 2013). According to Kumar *et al*. (2011), collagen derived from fish has a complex structural protein and a smaller structure compared to collagen made from land animals. Hence, it is easily absorbed by the human body. One of fish with skin that has good chemical content and has the potential to be a source of collagen production is Alaska Pollock (*Theragra chalcogramma*). According to the Food and Agriculture Organization (FAO, 2017), Alaska Pollock has a high production value for surimi and fillet products, as evidenced by data on marine aquaculture production of Alaska Pollock fish in 2014 reaching 3,245 tons, while in 2015 there was an increase in production of 3,372 tons. The data explains that the skin waste fillet industry of the Alaska Pollock fish showed an increase in production. In addition, the skin of the Alaska Pollock has a potential to be used as a raw material for producing collagen to increase value of by-product from the fish fillet industry.

Nowadays, isolation process of collagen could be done using several techniques. Fish skin isolation techniques are generally extracted by acid (ASC) (Pati *et al*., 2010; Van De Water *et al*., 2013; Liang *et al*., 2014; Chen *et al*., 2016), alkali and enzyme (PSC) (Pati *et al*., 2010; Liang *et al*., 2014; Wu *et al*., 2019) methods. These techniques are capable of producing collagen with a high level of purity but requires longer production time, large number of chemicals and high production costs, causing the produced collagen unable to compete with commercial collagen. Another technique that is considered to require low production costs and can produce collagen in a relatively short time is the hydro-extraction method. The hydro-extraction technique is an isolation process using water as a catalyst (heat transfer medium) and temperature as pressure (High Temperature Short Time/HTST) (Kolanus *et al*., 2019). According to Huang *et al*. (2016), hydro-extraction technique has several advantages, including shorter production time, lower production costs, high yield yields with easy controlling number of waste, which supports clean technology and sustainable production. Moreover, when compared to alkaline or acid soluble collagen, the collagen produced is water-soluble and safe for long-term consumption. Based on these problems, information regarding variations in the concentration of acetic acid for pre-treatment process to manufacture water-soluble collagen from Alaska Pollock fish skin using the hydro-extraction method has never been reported. Hence, it is necessary to conduct research for water

collagen production by using hydro-extraction with pre-treatment at different concentrations of acetic acid in which the water-soluble collagen from Alaska Pollock fish skin using the hydro-extraction has never been reported. Oslan *et al.* (2022) reported that acid solubilization or using acetic acid extraction is the best method for producing collagen. Acid can break crosslink in the collagen helix which could be resulting high yield of collagen. The aim of this study was to evaluate based on the effect of the acetic acid concentration variation of in the pre-treatment step to produce water-soluble collagen Alaska Pollock fish skin via hydro-extraction method.

2. Material and Methods

2.1 Material

The materials used in this study was Alaska Pollock fish skin obtained from PT. Kelola Mina Laut in Gresik, East Java. Distilled water, sodium hydroxide (NaOH) (MERCK), acetic acid (CH_3COOH) (MERCK), boric acid ($H_3(BO_3)$, methylene red ($C_{15}H_{15}N_3O_2$), methylene blue ($C_{16}H_{18}N_3SCI$), ethanol (MERCK), N-hexane (C_6H_{14}) (MERCK), hydrochloric acid (HCl) (MERCK), sulfuric acid (H_2SO_4) (MERCK), Kjeldahl tablets, aquabides, AccQ Fluor Borate solution, 20 µL AccQ• Tag Ultra Derivatization Kit for amino acid standard- Waters Corporation.

2.2 Method

2.2.1 Preparation of fish skin of Alaska Pollock (Theragra chalcogramma)

The Alaska Pollock fish skin sample was washed using running water to remove the undesirable component. The remaining meat, scales and fat in the skin were then removed using a sharp object. The samples' skins then underwent an organoleptic test (9-point hedonic scale) to examine the freshness and quality of the skin. Later, all parts of the Alaska Pollock fish skins were cut into small pieces with appropriate thickness with dimensions ranging 1x1 cm^2. The samples of Alaska Pollock fish skin were then stored in freezer until used for the further experiment.

2.2.2 Deproteinization process

The Alaska Pollock fish skin samples were washed and then weighed before entering the deproteinization stage. The deproteinization process was carried out by immersing the skin with 0.05M of NaOH with a 1 to 10 ratio (w/v) at chilling temperature and shaken with magnetic stirrer. The aim of the process was to remove non-collagen proteins. Every hour of the three-hour soaking period, the NaOH solution was replaced by a fresh solution. The samples were then washed with distilled water until a neutral pH was reached.

2.2.3 Pre-treatment of water-soluble collagen

The samples from the deproteinization process were then used for the pre-treatment with acetic acid to produce water-soluble collagen. The samples were immersed in different concentrations of acetic acid (0.01 M; 0.05 M; 0.1 M; and 0.15 M) with a 1 to 10 ratio of skin with acetic acid solution (w/v) for two hours. After soaking with acetic acid, the Alaska Pollock fish skin sample was washed using distilled water until a neutral pH was reached. The samples were then stored in freezer until used for further experiment.

2.2.4 Water-soluble collagen production

The samples skin of Alaska Pollock from the previous result was then used for hydro-extraction process. The hydro-extraction process was carried out at a temperature of 40°C for two hours, with a rpm of 150 in an incubator shaker. The ratio of skin and distilled water was 1 to 1 (w/v). The water-soluble collagen was stored in the refrigerator for 24 hours to form a gel. The samples were then dried using a freeze dryer to obtain a water-soluble collagen in powder form. The sample was then stored in room temperature for further analysis.

2.2.5 Amino acid analysis

Amino acid analysis was performed using HPLC. The water-soluble collagen extracts were diluted in 6 N HCl and went through hydrolysis in boiling water bath at 110°C for a period of 24 hours. The samples were shaken or swirled every one hour to get proper hydrolysis process. The samples were then centrifuged at 3,500 rpm for 15 minutes. The supernatant was filtered and neutralized with 1 N NaOH. The filtered solution was then diluted to 1:100 of volume (1 ml diluted to 100 ml) with distilled water and loaded onto HPLC. The amino acid properties were calculated as part per million of protein (Gratzfeld-Huesgen, 1998).

2.2.6 Yield

The yield of water-soluble collagen was calculated based on freeze-dried sample (Normah *et al.*, 2018) by the following equation:

$$\text{Collagen yield (g/100 g)} = \frac{\text{weight of lyophilized collagen}}{\text{weight of initial dry fish pretreated byproduct}} \times 100\% \quad \ldots \text{Eq (1)}$$

2.2.7 Proximate analysis (Moisture, ash, crude protein, lipid content)

The water-soluble collagen was determined

by the proximate analysis based on standard method (AOAC, 2007). Moisture was determined by a moisture analyzer (BEL i-Thermo 163L, India), ash content was analyzed by ashing by placing the sample in a furnace with a temperature 550°C until the sample turned to ash with grey color, and crude protein content was analyzed using Kjeldahl method including destruction, distillation, and titration. Whereas the total of lipid was characterized by using Soxhlet methods. Then, the collected data were calculated on a dry basis of water-soluble collagen.

2.3 Analysis Data

This study was used to design a completely randomized design (CRD) with four treatments and three replications, 12 study units were found with different concentrations CH_3COOH, namely 0.01, 0.05, 0.1, and 0.15 M. The research layout to determine different concentration of acetic acid concentration on water-soluble collagen (WSC) was analyzed with one element ANOVA using Microsoft Excel program version 16.59. These methods were used to analyze the effect of different concentrations of acetic acid against proximate analysis and yield.

3. Results and Discussion

Collagen is the major structure in connective tissues such as a tendon, skin, and bone. Collagen has an isoelectric point near physiological pH, which means it does not dissolve in neutral pH solution. These properties affected the limitation of application in many fields such as medical, cosmetic, or injectable material (Friess and Lee, 1996; Nezu and Winnik, 2000; Sripriya *et al*., 2011; Patino *et al*., 2022). In this research we tried to produce water-soluble extraction by using hydro-extraction technique with pre-treatment using acetic acid. In first stage, fish skin preparation was carried out (Figure 1). To determine the freshness and quality of the skin, an organoleptic test was carried out. Organoleptic test of Alaska Pollock fish skin was carried out with the parameters of appearance, smell, and texture in line with SNI 01-2729-1992. The test was carried out by 30 untrained panelists to provide an assessment in the formula based on the available criteria and specifications.

Based on the findings of the average organoleptic test calculation, a score of 9 was obtained for the appearance and odor parameters, while a score of 8.33 was obtained for the texture parameter (Figure 2). According to Indonesian national standard SNI 01-2729-1992, fresh fish has an average score of 7, indicating that Alaska Pollock fish skin was suitable for usage as a raw material for collagen. The appearance of skin of the Alaska Pollock fish was bright, transparent, and has a clearly curved lateral line that was present from the head to the tail, the skin on the belly was grayish white and the skin on the back was brown. The skin of Alaska Pollock fish has a distinctive smell and has an elastic texture.

In general, acetic acid concentration pre-treatment had a significant effect ($p<0.05$) on the proximate composition, pH, and yield of water-soluble collagen produced, resulted in a variation of water content (8.02-11.08%), ash content of (0.08-0.98%), protein content of (85.52- 91.13%), fat content of (0.06-0.20%), pH of (6.99-7.12), and yield of (2.29-3.13%) (Table 1). The characterization of water-soluble collagen from skin of Alaska Pollock (*Theragra chalcogramma*) (Table 1) met the criteria of the National Standards Indonesia SNI 8076:2014). Luo *et al*. (2018) reported that proximate analysis of skin collagen from Siberian sturgeon was a water content of (68.97 ± 3.06 g 100 g^{-1}), ash content of (9.64 ± 0.38 g 100 g^{-1}), protein content of (15.24 ± 1.17 g 100 g^{-1}), and fat content of (0.41 ± 0.03 g 100 g^{-1}) on a wet weight basis. The difference of proximate analysis was due to variance in fish skin and collagen extraction. In this study, the extraction process was done by pre-treatment with acetic acid, neutralization, and hydro-extraction to produce collagen. Consequently, the fat content of collagen was low and had a low ash content with high protein. The high protein content of collagen indicated that the extraction process to remove impurities of Alaska Pollock fish skin was done effectively.

The water content in a material can determine the quality of the material because it relates to shelf life, freshness, and food safety. High water content can also affect metabolic activities such as enzyme activity, microbial activity, and chemical activity, causing changes in nutrient content and organoleptic properties (Rosida *et al*., 2018). The moisture content of each sample is closely related to the humidity of the sample, reflecting water content and affected sample texture (Harjo *et al*., 2015). The analysis of water content of water-soluble collagen met the criteria of Indonesian national standard. This result showed that the freeze drying process has been carried out according to the standard.

The ash content of a product provides information about its purity, mineral content, and cleanliness of the product (Kristiandi *et al*., 2021). The decreased total ash content is one of the parameters of collagen quality which means the extraction process were done optimally. Higher ash content in the sample showed that the extraction process was not done optimally because of

many mineral residues left behind (Khirzin *et al*., 2019). There is a correlation between the concentration of acetic acid and the percentage of ash content in which higher concentration of acid used more minerals are eliminated resulting in a lower percentage of ash content and vice versa (Suptijah *et al*., 2013). The obtained results also showed the same phenomenon with resulting low content of ash. This showed that the extraction process for making water-soluble collagen was successfully carried out via hydro-extraction method undergone with acetic acid pre-treatment step.

Protein content can be used as a parameter to determine the quality of the fish skin raw material. The results of high protein content means better quality of the raw material (Nugraheni *et al*., 2021). Alaska Pollock fish skin is known to have a high source of protein content. Li *et al*. (2018), reported that the protein content of Alaska Pollock fish skin was 85.46%. Therefore, Alaska Pollock fish skin is an ideal raw material for collagen production. The high protein content in the skin needed high concentration of acid level for demineralization process. Nugraheni *et al*. (2021) stated that acetic acid solution enters the skin cells and caused breakdown of hydrogen bonds in the peptide chains, while H^+ ions from an acidic solution made it easier for water to penetrate into the collagen fibers. This process was affected by swelling of the fish skin to produce procollagen structure thereby facilitating the extraction process.

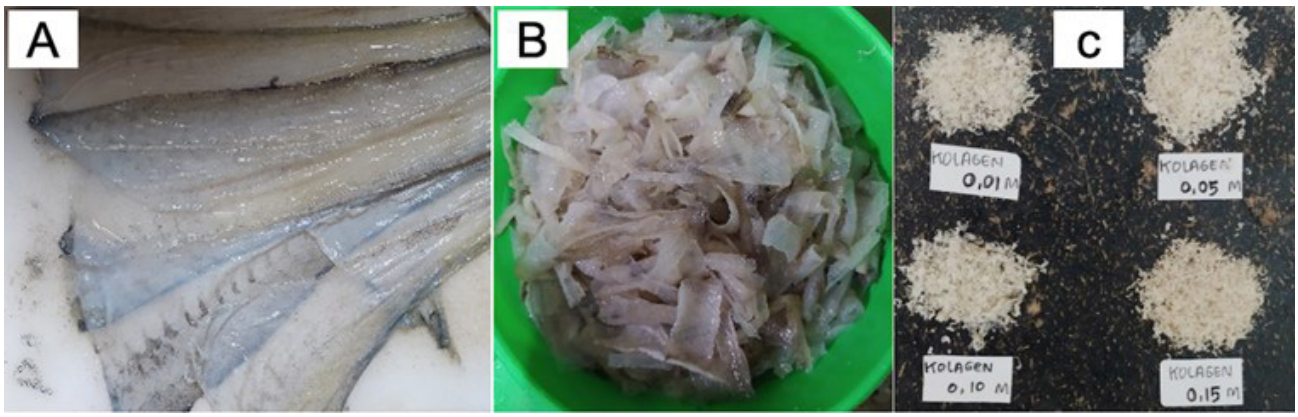

Figure 1. Morphological images of: (a) Alaska Pollock (*Theragra chalcogramma*) fish skin; (b) fish skin after deproteination and neutralization; (c) dried water-soluble collagen products pretreated with acetic acid at different concentrations (0.01 M; 0.05 M; 0.1 M, 0.15 M).

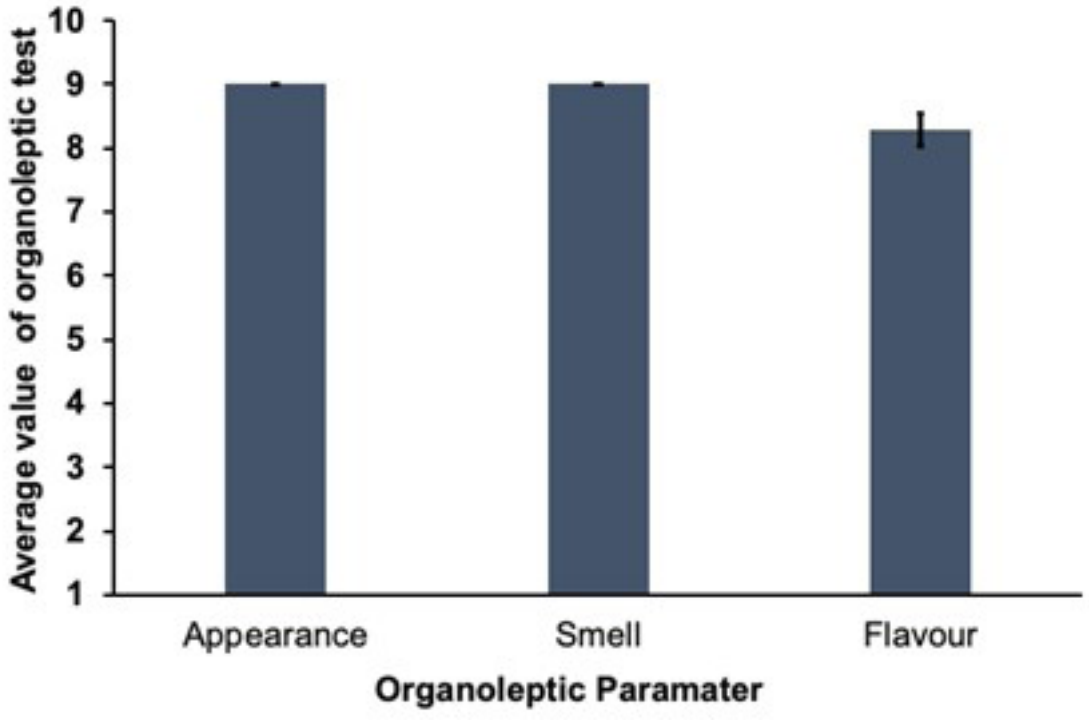

Figure 2. Average organoleptic score (on 9-point hedonic scale) Alaska Pollock (*Theragra chalcogramma*) skin raw material.

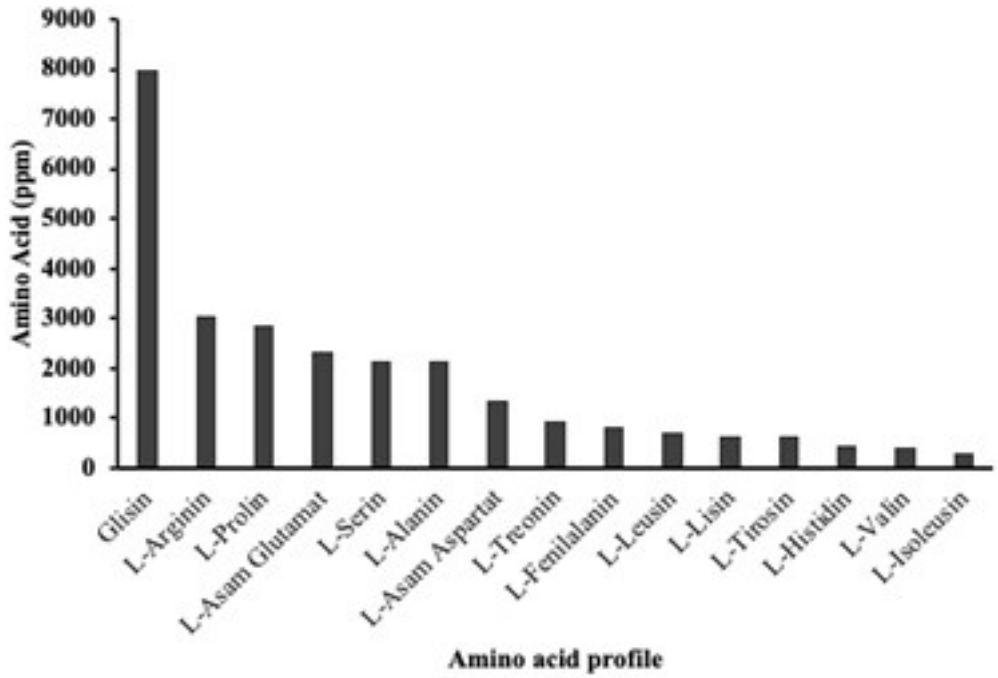

Figure 3. Amino acid composition of water-soluble collagen extracted from skin of Alaska Pollock Fish Skin (*Theragra chalcogramma*)

Table 1. Characterization of water-soluble collagen from skin of Alaska Pollock (*Theragra chalcogramma*)

Parameter	Acetic acid concentration				Indonesian National Standard
	0.01 M	0.05 M	0.10 M	0.15 M	
Water content (%)	11.08^{a}±0.01	8.07^{b}±0.01	11.02^{a}±0.01	8.02^{b}±0.07	≤12%
Ash content (%)	0.98^{a}±0.02	0.76^{b}±0.04	0.15^{c}±0.05	0.08^{c}±0.03	≤1%
Protein content (%)	86.61^{c}±0.05	87.35^{b}±0.01	85.52^{d}±0.03	91.13^{a}±0.05	≥75%
Lipid content (%)	0.20^{a}±0.02	0.15^{b}±0.03	0.10^{c}±0.01	0.06^{d}±0.01	≤1%
pH	7.12^{a}±0.03	7.08^{b}±0.02	7.03^{c}±0.01	6.99^{d}±0.01	6.5-8
Yield (%)	2.29^{d}±0.03	2.60^{c}±0.06	2.90^{b}±0.05	3.13^{a}±0.04	-

The lipid content of water-soluble collagen from Alaska Pollock fish is very important because lipid can affect the quality of collagen during storage. Lipid damage affects the nutritional value and cause a distortion of taste and smell. High-quality collagen was expected to have a low lipid content and expected to contain no fat (Hudzaifah, 2013). In addition, lipid content can be correlated with the pre-treatment of acetic acid. Higher concentration of acetic acid applied results in lower lipid content obtained in the collagen samples. The acetic acid has ability to breaking down of the bond structure in proteins and can bind with fat molecules (Nolsøe and Udeland, 2009). Therefore, in the neutralization process with distilled water, the fat will be removed, and causes reduced lipid content in the samples (Said *et al*., 2011). The research data showed characterization of water-soluble collagen from skin of Alaska Pollock (*Theragra chalcogramma*) (Table 1) and the appearance of dried water collagen (Figure 1). This is in line with research from Said *et al*. (2011) which stated that the lipid content met the criteria of National Indonesian Standard (SNI 8076:2014). This indicated that the hydro-extraction process was an effective method for removing the lipid content in the samples.

The pH value in water-soluble collagen from Alaska Pollock fish can be used as a parameter in determining collagen quality standards. This is because

the pH value can affect the viscosity and strength of the collagen gel (Said *et al*., 2017). The pH value can be affected with the use of acetic acid concentration and neutralization process after soaking with acids or bases. The combination of the soaking process with acids and bases tends to produce a pH value that is close to neutral. The higher the concentration of acetic acid used, the lower the pH value, this is because acetic acid diffuses more into the fish skin tissue. Hence, during the skin washing process, more acetic acid was left behind. In addition, the neutralization process carried out will also affect the final pH of collagen. The results showed that the water-soluble collagen has a neutral pH (Table 1). However, further research is needed regarding the solubility and other biological activities. The current data only showed that hydro-extraction is an alternative method that can be used for collagen pro duction which can reduce the use of chemicals.

The effectiveness and efficiency of the extraction technique affacted the yield value when producing water-soluble collagen from Alaskan Polloc fish. High yield suggested that the techniques employe and the treatments used were successful and effectiv (Febriansyah *et al*., 2019). The results showed that th yield of dry base collagen from Alaska Pollock fis skin extracted by hydro-extraction was still low (2.29-3.13%). This is because the concentration of acetic aci used to hydrolyze collagen influences the yield. Anothe research reported that to extract collagen by water acidified with CO_2 from skin Atlantic cod (*Gadus morhua*) showed a total yield of 13.8% (w/w) (Sousa *et al*., 2020). Kiew and Don (2013) reported that yield of collagen was increased when acetic acid reached 0.7 M and reverse trends with a decreased yield when acetic acid reached 0.9 M. In addition, Rosida *et al*. (2018) reported that the amount of collagen yield produced increased, indicating that the concentration of acetic acid solution has a significant effect on extraction of collagen. This report also supported by Mulyani *et al*. (2017) whic stated that as the acid concentration increased the H^+ and caused more cross-links in the helical side telopeptide area that is split, a collagen that is easier to dissolve. In addition, Paudi *et al*. (2020) reported that the use o acetic acid has not been able to completely break down the fibrils and is classified as a weak acid. The low concentration of H^+ ions in system extraction. also affected by acetic acid, caused the lack of maximum solubility of collagen in acetic acid. The solubility of collagen at the pre-treatment step would be affected by the yield of final product which is water-soluble collagen. According to Kiew and Don (2013) acetic acid solution has a key role in extraction of collagen and Giménez *et al*. (2005) stated that increase in H^+ ion cause a more effective water access to collagen fiber because electrostatic swelling or lyotropic hydration happens in the water. Therefore, concentration of acetic acid with high concentration would affect swelling properties/solubilization of collagen. Based on this information, increasing the yield of water-soluble collagen need further experiment such as increasing concentration of acetic acid in pre-treatment, combination of physical or enzymatic process and/ combination process between two or more extraction methods of water-soluble collagen.

Based on the results of the amino acid analysis using HPLC showed that the predominate amino acids in collagen were glycine, arginine, proline, glutamic acid, serine, and alanine (Figure 3). The results are in line with previous report by Hema *et al*. (2013) which stated that the amino acid composition of collagen tends to be dominated by glycine, proline, and alanine. In addition, collagen must have low levels of tyrosine and histidine (Aberoumand, 2012). Collagen contains high amounts of the amino acids of glycine, alanine, proline, and contains little of the amino acids of histidine and tyrosine, and does not contain cystine, which are characteristic of type I collagen (Nalinanon *et al*., 2011). Type I is the most important type of collagen in the body. Type I collagen is collagen has a function as a constituent material on skin formation, bone, tendons, cartilage, connective tissue, and teeth as well as acting as an extracellular protein matrix with characteristics of increasing cell proliferation. Therefore, it could affect cell physiology and morphology. Fawzya *et al*. (2016) reported that factors including raw materials used, extraction methods, different concentrations of extractive materials and methods of amino acid analysis and differences in the types of sample materials tested could affect properties of amino acid. The samples for analysis of amino acid used wet water-soluble collagen. This process affected the value of amino acid detected by HPLC which was relatively low due high content of water. Therefore, further analysis for amino acid is recommended for usage of samples in dry or powder form.

4. Conclusion

Based on the results of the study, the characteristics of water-soluble collagen products extracted from Alaska Pollock (*Theragra chalcogramma*) fish skin through a pre-treatment with different concentrations of acetic acid showed that the proximate compositions met the criteria of Indonesian National Standard (SNI 8076:2014). The optimal pre-treatment to produce water-soluble collagen from Alaska Pollock fish skin was using 0.15 M of acetic acid resulting in the highest yield

(3.13%) and protein content (91.13%). It could be concluded that that higher acetic acid concentration at a pre-treatment step enhanced the yield of water-soluble collagen.

Acknowledgement

The authors are grateful for funding and research facilities provided by Universitas Airlangga No:810/UN3.15/PT/2021.

Authors Contribution

The contribution of each author is as follow, Patmawati, Aliffiansyah Rizky Ergion; collected data, analyzed, and wrote the article. Patmawati, Laksmi Sulmartiwi, Dwita Nirmala, Sapta Wijayanti, Raseetha Siva, and Yaowapha Waiprib; supervised, devised the main conceptual ideas, wrote the article, critically revised the article. All authors discussed the results and contributed to the final manuscript.

Conflict of Interest

The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Funding Information

The research was funded by Universitas Airlangga, with grant number of 810/UN3.15/PT/2021.

References

Aberoumand, A. (2012). Comparative study between different methods of collagen extraction from fish and its properties. *World Applied Sciences Journal*, 16(3):316-319.

AOAC. (2007). Official methods of analysis (17th ed.). Washington, DC: Association of Official Analytical Chemists.

Chen, J., Li, L., Yi, R., Xu, N., Gao, R., & Hong, B. (2016). Extraction and characterization of acid-soluble collagen from scales and skin of tilapia (*Oreochromis niloticus*). *LWT- Food Science and Technology*, 6:453-459.

Chen, S., Hong, Z., Wen, H., Hong B., Lin, R., Chen, W., Xie, Q., Le, Q., Yi, R., & Wu, H. (2022). Compositional and structural characteristics of pepsin-soluble type I collagen from the scales of red drum fish, *Sciaenops ocellatus*. *Food Hydrocolloids*, 123:107111.

Fawzya, Y. N., Chasanah, E., Poernomo, A., & Khirzin, M. H. (2016). Isolasi dan karakterisasi parsial kolagen dari teripang gamma (*Sticopus variegatus*). *Jurnal Pascapanen dan Bioteknologi Kelautan dan Perikanan*, 11(1):91-100.

Febriansyah, R., Pratama, A., & Gumilar, J. (2019). Pengaruh konsentrasi NaOH terhadap rendemen, kadar air dan kadar abu gelatin ceker itik (*Anas platyrhynchos Javanica*). *Jurnal Ilmu dan Teknologi Hasil Ternak*, 14(1):1-10.

Food and Agriculture Organization (FAO). (2017). FAO global capture production database updated to 2015 - summary information. Italy: Food and Agriculture Organization of United Nations.

Friess, W., & Lee, G. (1996). Basic thermoanalytical studies of insoluble collagen matrices. *Biomaterials*, 17(23):2289-2294.

Giménez, B., Turnay, J., Lizarbe, M. A., Montero, P., & Gómez-Guillén, M. C. (2005). Use of lactic acid for extraction of fish skin gelatin. *Food Hydrocolloids*, 19(6):941-950.

Gratzfeld-Huesgen, A. (1998). Sensitive and reliable amino acid analysis in protein hydrolysates using the Agilent 1100 series HPLC. *Hewlett Packard Technical Note*, 12:5966-3110.

Harjo, S. S. T., Lilik, E. R., & Djalal, R. (2015). Perbandingan madu karet dan madu rambutan berdasarkan kadar air, aktivitas enzim diastase dan hidroximetilfurfural (HMF). *Jurnal Ilmu dan Teknologi Hasil Ternak*, 10(1):18-21.

Hema, G. S., Shyni, K., Mathew, S., Ananda, R., Ninan, G., & Lakshmanan, P. T. (2013). A simple method for isolation of fish skin collagen-biochemical characterization of skin collagen extracted from albacore tuna (*Thunnus alalunga*), dog shark (*Scoliodon sorrakowah*), and rohu (*Labeo rohita*). *Annals of Biological Research*, 4(1):271-278.

Huang, C. Y., Kuo, J. M., Wu, S. J., & Tsai, H. T. (2016). Isolation and characterization of fish scale collagen from tilapia (*Oreochromis* sp.) by a novel extrusion-hydro-extraction process. *Food*

Chemistry, 190:997-1006.

Hudzaifah, M. R. (2013). Karakterisasi sifat fisikokimia gelatin kulit ikan sapu-sapu (*Hyposarcus pardalis*) hasil hidrolisis asam. Jakarta: Universitas Islam Negeri Syarif Hidayatullah.

Jafari, H., Lista, A., Siekapen, M. M., Ghaffari-Bohlouli, P., Nie, L., Alimoradi, H., & Shavandi, A. (2020). Fish collagen: Extraction, characterization, and applications for biomaterials engineering. *Polymers*, 12(10):1-37.

Kementerian Kelautan dan Perikanan (KKP). (2013). Statistik kelautan dan perikanan dalam angka 2013. Jakarta: Kementrian Kelautan dan Perikanan.

Khirzin, M. H., Ton, S., & Fatkhurrohman. (2019). Ekstraksi dan karakterisasi gelatin tulang itik menggunakan metode ekstraksi asam. *Jurnal Sain Peternakan Indonesia*, 14(2):119-127.

Kiew, P. L., & Don, M. M. (2013). The influence of acetic concentration on the extractability of collagen from the skin of hybrid *Clarias* sp. and its physicochemical properties: a preliminary study. *Focusing on Modern Food Industry*, 2(3):123-128.

Kolanus, J. P. M., Sugeng, H., & Syarifuddin, I. (2019). Karakterisasi kolagen larut asam dari kulit ikan tuna (*Thunnus albacores*) dengan metode hidroekstraksi. *Jurnal Riset Teknologi Industri*, 13(1):99-110.

Kristiandi, K., Rozana, J., & Andi, M. (2021). Analisis kadar air, abu, serat dan lemak pada minuman sirop jeruk siam (*Citrus nobilis microcarpa*). *Jurnal Keteknikan Pertanian Tropis dan Biosistem*, 9(2):165-171.

Kumar, M. H., Spandana, V., & Poonam, T. (2011). Extraction and determination of collagen peptide and its clinical importance from tilapia fish scales (*Oreochromis niloticus*). *International Research Journal of Pharmacy*, 2(10):97-99.

Kusumawinahyu, C. A., Abidin, S. H., Patmawati, Pudjiastuti, D. Y., Dwitha Nirmala, D., Alamsjah, M. A., Sulmartiwi, L., & Raseetha, S. (2022). Effects of different acetic acid immersion time on the properties of collagen from pangasius skin. *Jurnal Ilmiah Perikanan dan Kelautan*, 14(2):411-417.

Li, Y., Chen, F., Zou, J., Zhi, M., & Huang, G. (2018). Gelatin extraction of alaska Pollock (*Theragra chalcogramma*) dry skin. *American Journal of Biochemistry and Biotechnology*, 14(1):13-19.

Liang, Q., Wang, L., Sun, W., Wang, Z., Xu, J., & Ma, H. (2014). Isolation and characterization of collagen from the cartilage of Amur sturgeon (*Acipenser schrenckii*). *Process Biochemistry*, 49(2):318-323.

Luo, Q. B., Chi, C. F., Yang, F., Zhao, Y. Q., & Wang, B. (2018). Physicochemical properties of acid-and pepsin-soluble collagens from the cartilage of Siberian sturgeon. *Environmental Science and Pollution Research*, 25:31427-31438.

Maroušek, J., Maroušková, A., Myšková, K., Váchal, J., Vochozka, M., & Žák, J. (2015). Techno-economic assessment of collagen casings waste management. *International Journal of Environmental Science and Technology*, 12(10):3385-3390.

Mulyani, S., Setyabudi, F. M. C. S., Pranoto, Y., & Santoso, U. (2017). Physicochemical properties of gelatin extracted from buffalo hide pretreated with different acids. *Food Science of Animal Resources*, 37(5):708-715.

Muralidharan, N., Shakila, R. J., Sukumar, D., & Jeyasekaran, G. (2013). Skin, bone, and muscle collagen extraction from the trash fish, leather jacket (*Odonus niger*) and their characterization. *Journal of Food Science and Technology*, 50(6):1106-1113.

Nalinanon, S., Benjakul, S., Kishimura, H., & Osako, K. (2011). Type I collagen from the skin of ornate threadfin bream (*Nemipterus hexodon*): Characteristics and effect of pepsin hydrolysis. *Food Chemistry*, 125(2):500-507.

Nezu, T., & Winnik, F. M. (2000). Interaction of water-soluble collagen with poly(acrylic acid). *Biomaterials*, 21(4):415-419.

Nolsøe, H., & Undeland, I. (2009). The acid and alkaline solubilization process for the isolation of muscle proteins: state of the art. *Food and Bioprocess Technology*, 2(1):1-27.

Normah, I., & Afiqah, M. M. (2018). Effect of extraction

time on the physico-chemical characteristics of collagen from sin croaker (*Johniecop sina*) waste. *International Food Research Journal*, 25(3):1074-1080.

Nugraheni, A. W., Anggo, A. D., & Dewi, E. N. (2021). Pengaruh jenis asam terhadap karakteristik gelatin kulit ikan ayam-ayam (*Abalistes stellaris*). *Jurnal Ilmu dan Teknologi Perikanan*, 3(2):78-85.

Oslan, S. N. H., Li, C. X., Shapawi, R., Mokhtar, R. A. M., Noordin, W. N. M., & Huda, N. (2022). Extraction and characterization of bioactive fish by-product collagen as promising for potential wound healing agent in pharmaceutical applications: Current trend and future perspective. *International Journal of Food Science*, 2022(9437878):1-10.

Pati, F., Adhikari, B., & Dhara, S. (2010). Isolation and characterization of fish scale collagen of higher thermal stability. *Bioresource Technology*, 101(10):3737-3742.

Patino, M. G., Neiders, M. E., Andreana, S., Noble, B., & Cohen, R. E. (2022). Collagen: an overview. *Implant Dentistry*, 11(3):280-285.

Paudi, R., Sulistijowati, R., & Mile, L. (2020). Rendemen kolagen kulit ikan bandeng (*Chanos chanos*) segar hasil ekstraksi asam asetat. *Jambura Fish Processing Journal*, 2(1):21-27.

Silvipriya, K.S., Kumar, K.K., Bhat, A.R, Kumar, B.D., John, A., Lakshmanan, P. 2015. Collagen: animal sources and biomedical application. *J. Appl. Pharm. Sci*. 5(3): 123–127.

Rosida, R., Handayani, L., & Dwi, A. (2018). Pemanfaatan limbah tulang ikan kambing-kambing (*Abalistes stellaris*) sebagai gelatin menggunakan variasi konsentrasi CH3COOH. *Acta Aquatica Aquatic Sciences Journal*, 5(2):93-99.

Said, M. I., Hajrawati, H., & Munda, M. (2017). Evaluasi Sifat-sifat kolagen tulang broiler pada penerapan kombinasi proses berbeda. *Jurnal Ilmu dan Teknologi Hasil Ternak*, 12(2):89-96.

Said, M. I., Triatmojo, S., Erwanto, Y., & Fudholi, A. (2011). Karakteristik gelatin kulit kambing yang diproduksi melalui proses asam basa. *Agritech*, 31(3):216-455.

Singh, P., Benjakul, S., Maqsood, S., & Kishimura, H. (2011). Isolation and characterization of collagen extracted from the skin of striped catfish (*Pangasianodon hypophthalmus*). *Food Chemistry*, 124(1):97-105.

Sousa R. O., Martins E., Carvalho D. N., Alves A. L., Oliveira, C., Duarte, A. R. C., Silva, T. H., & Reis, R. L. (2020). Collagen from Atlantic cod (*Gadus morhua*) skins extracted using CO_2 acidified water with potential application in healthcare. *Journal of Polymer Research*, 27(73):1-9.

Sripriya, R., Kumar, R., Balaji, S., Kumar, M. S., & Sehgal, P. K. (2011). Characterizations of polyanionic collagen prepared by linking additional carboxylic groups. *Reactive and Functional Polymers*, 71(1):62-69.

Suptijah, P., Suseno, S. H., & Cholil, A. (2013). Analisis kekuatan gel (gel strength) produk permen jelly dari gelatin kulit ikan cucut dengan penambahan karaginan dan rumput laut. *Jurnal Pengolahan Hasil Perikanan Indonesia*, 16(2):183-191.

Van De Water, L., Varney, S., Tomasek, J. J. (2013). Mechanoregulation of the myofibroblast in wound contraction, scarring, and fibrosis: Opportunities for new therapeutic intervention. *Advances in Wound Care*, 2(4):122-141.

Walters, B. D., & Stegemann, J. P. (2014). Strategies for directing the structure and function of three-dimensional collagen biomaterials across length scales. *Acta Biomaterialia*, 10(4):1488-1501.

Wu, J., Guo, X., Liu, H., & Chen, L. (2019). Isolation and comparative study on the characterization of guanidine hydrochloride soluble collagen and pepsin soluble collagen from the body of surf clam shell (*Coelomactra antiquata*). *Foods*, 8(1):1-13.