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Extraction of Gelatin from Salmon Skin

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Abstract: The primary source to produce gelatin is porcine and bovine. However, the usage of mammalian gelatin leads to a few diseases, such as bovine sponge encephalopathy, transmissible spongiform encephalopathy, and hand-foot-mouth disease. Besides, some religions restricted their believers to consume porcine and bovine gelatin, for instance, Muslim, Hindu, and Jewish. In this aspect, the polemic has cause anxiety to the public. Hence the demand for gelatin from non-mammals has been an uprising. Next, the production of gelatin from fish by-products is considered as a wise solution to this problem. Furthermore, the vast waste generated by fish after filleting, such as skin, scales, and bones, cause a great expense for disposal. Other than that, recycling aquatic by-products is one of the ways to save the environment. Therefore, the objectives of gelatin extraction from salmon skin are to extract type-A gelatin from salmon skin and determine the melting temperature. Then, the dry gelatin obtained will be weighed to calculate the yield percentage and compared it with mammalian gelatin. Consequently, the functional groups will be studied using FTIR analysis that indicates the gelatin content. The salmon skin gelatin will be extracted with controllable parameters in this experiment which are different pretreatment time (8, 16, and 24 hours) and hydrochloric acid concentration (0.05, 0.10, 0.50, 1.00 M). Results from the research focused shows that salmon skin gelatin has a lower yield percentage when the pretreatment time increase. The highest yield of 12.71 % was spotted at 0.50 M HCl during 8 hours. The expected result of bloom strength decrease as the pretreatment time increase and the concentration of HCl decrease. Besides, salmon skin gelatin possesses a lower melting temperature within the range of 10.7 °C to 11.5 °C. Amide I and amide II in FTIR analysis indicate the gelatin content. Thus, the type-A salmon skin gelatin successfully extracted.

Keywords: Salmon Skin, Gelatin, HCl, Pretreatment

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1. Introduction

Gelatin is a relatively low-priced food ingredient as it is typically generated as waste. It is plentiful since it can be obtained from a vast horizon of land and marine sources and has fantastic functional properties [1]. Besides, gelatin has numerous emerging applications in cosmetics, pharmaceuticals, biomedical, and biomaterial-based packaging industries. A couple of relevant factors need to be taken into account when choosing gelatin from fish, such as water-type species, types of fish, and fish components used. The physical properties of gelatin may be significantly impacted by fish's origin, either warm-water fish or cold-water fish. Skin gelatin from warm-water fish has a melting temperature similar to that of skin gelatin from warm-blooded animals [1].

In contrast, cold-water fish gelatin and warm-blooded animals show a different amino acid profile and gel strength. Cold-water fish gelatin has a lower concentration of amino acids (proline and hydroxyproline) and lower molecular weight distribution than mammalian gelatin. As a result, cold-water fish gelatin shows marked differences in thermal, rheological, viscoelastic, and mechanical properties compared to mammalian and warm-water fish [2].

Generally, gelatin processing includes three stages, beginning with the pre-treatment of raw materials, thermal extraction, and recovery processes, including evaporation and drying processes. There are two classification types: gelatin, type-A (extracted from acid-treated collagen), and type-B (extracted from alkali-treated collagen). Fish gelatin is commonly known as type-A gelatin since it has fewer crosslinked collagen. Collagen consists of three polypeptide chains intertwined and form a triple helix structure, which is stabilized by an intra-and inter-chain hydrogen bond. Suitable chemical treatment can create ample swelling and disrupt non-covalent bonds. It is possible to convert collagen to soluble gelatin by gluing hydrogen and covalent bonds that stabilize the triple helix shape into a coiled shape, resulting in soluble gelatin conversion [3].

Therefore, the purposes of this work are to extract type-A salmon skin gelatin, to study the functional properties of type-A gelatin extracted from salmon skin, including its Bloom strength and melting temperature, as well as to compare the yield of gelatin produced from fish skin to mammalian gelatin. Consequently, this work will help a fundamental understanding of the relationships between the properties and function of gelatin in the industries and provide useful knowledge on the value-added application of aquatic by-products.

2. Materials and Methods

2.1 Materials

The species used in this study was salmon fish. The frozen salmon fish skin was stored in the freezer at -20 °C until further use. Hydrochloric acid of Ar 37 % from Sigma Aldrich has been used in this study.

2.2 Methods

The frozen salmon skins were thawed in a basin filled with tap water for a few hours. Then, the attached meat and scales were removed from the skins. The skins were cleaned with tap water and cut into sizes of 3 to 4 cm. They were put in a water bath afterward for 30 minutes at 36 °C and notified into 12 samples. Each sample weighed approximately 50g. Next, the skins were immersed in 0.05, 0.10, 0.50, and 1.00 M of HCl solutions for 8, 16, and 24 hours. After soaking, the skins were washed with tap water repeatedly and rinsed with distilled water until the pH achieved 4 to 7. Consequently, the treated skins were transferred into a glass jar filled with distilled water and were put in a water bath for 8 hours for extraction purposes. The solutions obtained were filtered using a cheesecloth of 4 layers and dried in the oven for 48 hours. The percentage of extracted gelatin yield was calculated using the formula in Eq.1.

$$Yield\ of\ gelatin\,\% = \frac{Dry\ weight\ of\ gelatin\ (g)}{Wet\ weight\ of\ salmon\ skin\ (g)} \times 100 \qquad \text{Eq. 1}$$

3. Results and Discussion

3.1 Yield of Gelatin

The bar chart below shows that the percentage yield decrease when the pretreatment time increase. The highest percentage yield of 12.71 % was recorded at 8 hours pretreatment time with 0.50 M of hydrochloric acid. The lowest percentage yield of 3.63 % was notified at 24 hours pretreatment time with 1.00 M of hydrochloric acid. Subsequently, when the concentration of hydrochloric acid higher, the percentage yield shifted higher, starting from 0.05 until 0.50 M and become lower at 1.00 M. The highest yield of salmon skin gelatin, 19.6 %, was obtained by [4]. However, [5] emphasised that the lower value of yield n lower concentration was observed due to loss of extracted collagen during preparation steps or incomplete collagen hydrolysis during the extraction process. Overall, the yield percentage of salmon skin gelatin is the lowest compared to warm-water fish and mammalian gelatin. Porcine and bovine have approximately 30 % of proline and hydroxyproline content while 25 % for warm-water fish and 17 % for cold-water fish like salmon and cod [6].

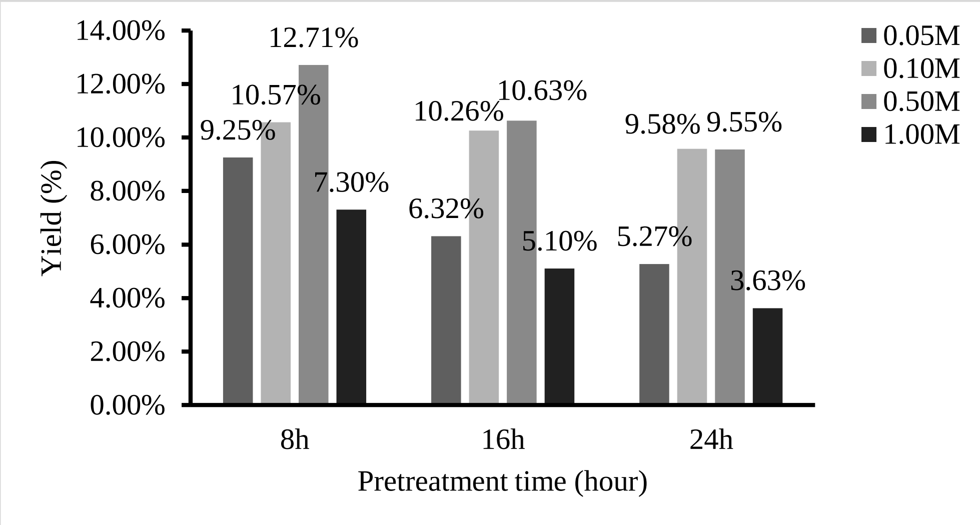

Figure 1: Percentage extracted yield of salmon skin gelatin

3.2 Fourier Transform Infrared (FTIR) Spectroscopy

FTIR spectra show the intensity for every peak locations of salmon skin gelatin. The spectra of 0.05 and 0.50 M for 8 and 16 hours portray quite a similar pattern in the Figure 2 below.

The amplitude of amide I in every sample were recorded to be between 1630 to 1634 cm^{-1}. Amide I peak is the coupling result of hydrogen bonding with COO meanwhile amide II indicate NH bending pair with CN stretching. In 16 hours pretreatment time, the amide I amplitude increase from 1632 to 1634 cm^{-1} as the concentration of hydrochloric acid increase [7].

Besides, for the hydrochloric acid concentration of 0.50M in 8 and 16 hours pretreatment time, the amplitude shifted from 1631 to 1634 cm^{-1}. [8] stated that the longer extraction caused a more significant loss of molecular order due to the thermal uncoupling of intermolecular crosslinks from collagen to gelatin. Other than that, the wavenumbers of amide II bands were noticeable at 1520 until 1524 cm^{-1} which occurs due to the bending of NH and the stretching vibration of CN.

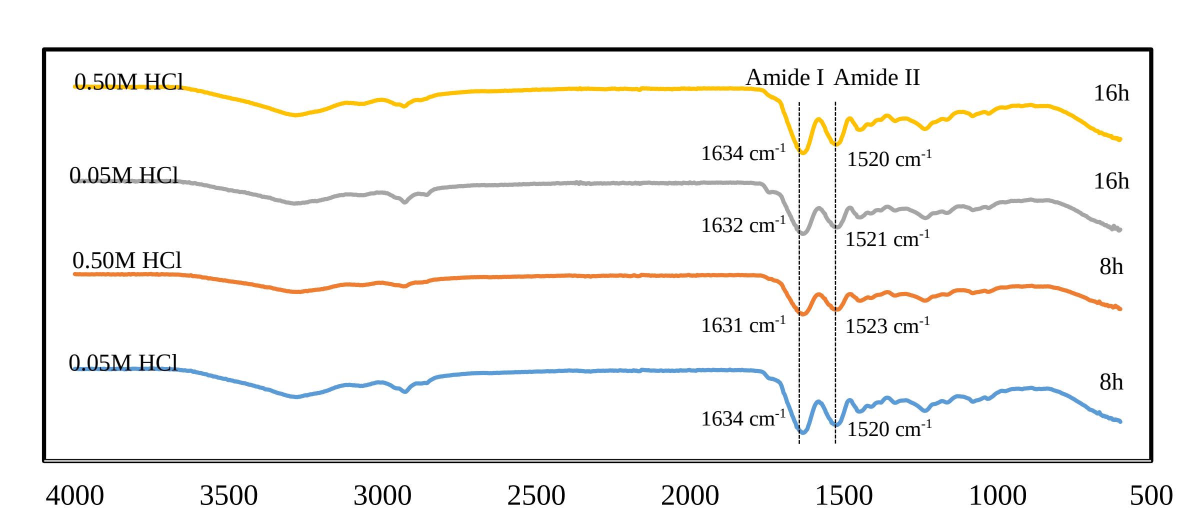

Figure 2: FTIR spectra of salmon skin gelatin at 8 hours pretreatment time

3.3 Melting Temperature

Based on the experiment conducted, the melting temperature of salmon skin gelatins were detected to be in the range of 10.7 °C to 11.5 °C. According to [9], the difference content of proline and hydroxyproline in collagens of various species relates to the temperature of the habitat of the animals. The melting temperature derived from salmon skin gelatin is lower than mammalians gelatin and warm-water fish gelatin since salmon is a cold-water fish. The lower the content of proline and hydroxyproline, the lower the melting temperature.

Figure 3 below shows that sample pre-treated at 8 hours in 0.50 M hydrochloric acid has the highest melting temperature which is 11.5 °C. Even though salmon skin gelatin posses a lower melting temperature, research by [10] justified fish gelatin in the lower range of 10 °C to 30 °C had excellent film forming properties that are suitable for production of vitamin capsules.

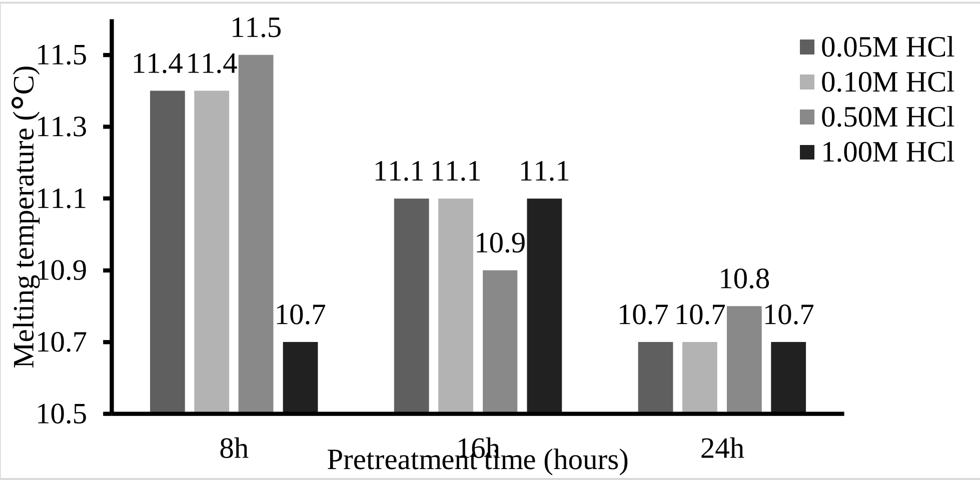

Figure 3: Melting temperature obtained from salmon skin gelatin

3.4 Bloom strength

Bloom or gel strength of fish gelatin usually ranging between zero to 270 compared to porcine and bovine, which have bloom value 200 to 240 [6]. From the bar chart, the expected bloom strength varied considerably with the acid treatment used for the swelling process of salmon skin. The bloom value decreases when the pretreatment time and concentration of hydrochloric acid increase. The most excellent (140 g) bloom strength is at 0.05 M in 8 hours and the weakest (51 g) at 1.00 M in 24 hours pretreatment time. It is assumed that the more extended storage and pH value influence the difference in bloom values after the preparation of the sample.

Bloom strength, which is below 150, is considered low but usually suitable for fruit juice products as a clarifying agent [11]. The expected Bloom value shown in Figure 4 is on par with the typical bloom value reported for cod, Alaska Pollock, salmon, and hake, 70 to 110. The differences in proline and hydroxyproline content affect the bloom strength. Lower hyroxyproline content means lower involvement of gel formation through hydrogen bonding by a hydroxyl group [12].

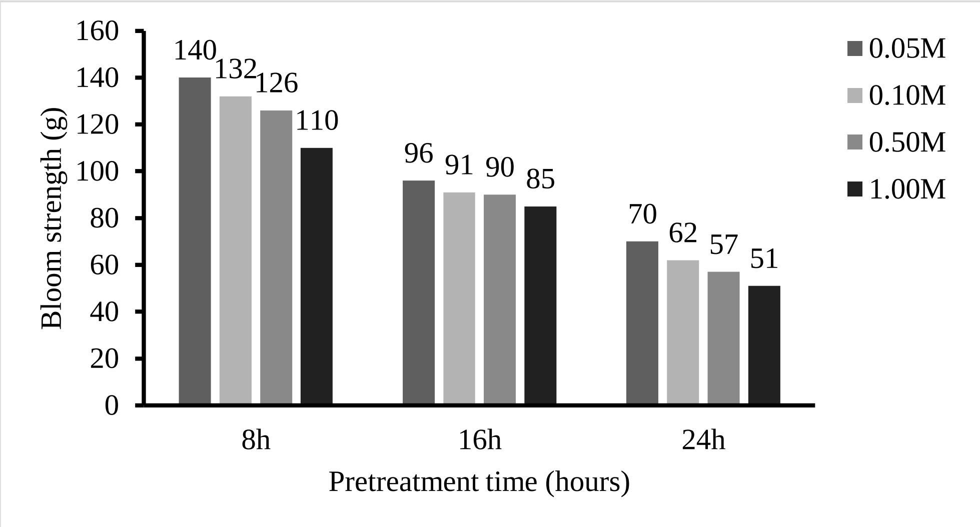

Figure 4: Expected bloom strength of salmon skin gelatin

4. Conclusion

In conclusion, the results presented consist of salmon skin gelatin yield, melting temperature, bloom strength, and functional groups of gelatin content via Fourier Transform Infrared Spectroscopy (FTIR) analysis showed that type-A gelatin was successfully extracted from salmon skin. Besides, the FTIR analysis revealed amide I and amide II in the gelatin content. The wavelengths of amide I can be detected in the range of 1630 until 1634cm^{-1}. Meanwhile, peak locations of amide II were notified at 1520 to 1524 cm^{-1}. Salmon skin gelatin also possessed a lower melting temperature range, 10.7 °C to 11.5 °C, due to the lower content of proline and hydroxyproline. Subsequently, the type-A salmon skin gelatin has a low but desirable gelatin yield, 12.71 %, in 0.50 M HCl concentration at 8 hours pre-treatment time. Next, the extracted salmon skin gelatin attributed to lower bloom values within the scope of 51 g to 140 g. In this case, cold-water fish tend to possess a lower Bloom strength compared to mammalian gelatin and warm-water fish. The origins of the fish, the content of proline and hydroxyproline, and the method and process of extraction are the factors that affect the results obtained.

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