Research Progress in Mechanical and Manufacturing Engineering Vol. 4 No. 1 (2023) 270-276

RPMME

Homepage: http://penerbit.uthm.edu.my/periodicals/index.php/rpmme
e-ISSN: 2773 - 4765

The Effect of Hydrochloric Acid to The Extraction of Gelatin from Salmon Skin

Nurdiana Azlan Hisham[1], Hasan Zuhudi Abdullah[1]*

[1]Faculty of Mechanical and Manufacturing Engineering,
Universiti Tun Hussein Onn Malaysia 86400 Parit Raja, Batu Pahat, Johor, MALAYSIA

*Corresponding Author Designation

DOI: https://doi.org/10.30880/rpmme.2023.04.01.030
Received 25 August 2022; Accepted 31 January 2023; Available online 01 June 2023

Abstract: Gelatin is generated from animal collagen that is mainly from mammalian' bones. The sources of gelatin frequently do not match the halal standard due to religious considerations. Therefore, fish gelatin has been discovered as an alternative to mammals' gelatin. This study aimed to produce gelatin from salmon skin, determine the yield at different acid concentrations and times, and identify the properties of the gelatin from salmon skin. The gelatin was extracted from salmon skin at various hydrochloric acid (HCl) concentrations (0.05M, 0.1M, 0.5M, and 1.0M) and times (6 hours, 18 hours, and 24 hours) followed by water extraction (50℃, 4 hours) and drying (60℃, 24 hours). The yield of the gelatin at 1.0M (6 hours) is the highest with 38.29%. The possible interactions in the functional group of gelatins were analyzed via Fourier Transform Infrared Spectroscopy (FTIR). Gelatin treated at a high concentration for a short length of time yields more gelatin and is thus appropriate for industrial application.

Keywords: Fish Gelatin, Hcl, Pre-Treatment, Salmon, Skin,

1. Introduction

Gelatin is generated from collagen, which is a naturally occurring structural protein found mostly in animal connective tissues [5]. Collagen is generally found in connective tissues, skin, cartilages, tendons, and bones [2] [13] [1] [10] [16] from animals which are the pig, bovine (beef or cattle) and fish.

Types of gelatin obtained from the collagen are dependent on the method of extraction which is type A (acid hydrolysis) and type B (basic hydrolysis) [7] [15]. These processes could result in the familiar characteristics of gelatin products that have lower molecular weight, are soluble in cold water, and do not form gels [11]. Gelatin is generally in powder form that is transparent, colorless, and almost tasteless. It is widely used in the food, pharmaceutical, and cosmetic industries as a gelling agent [7].

The usage of gelatin has been increased in various industries concerning most people as mostly the production of gelatins is from mammals which are generally porcine and bovine hide as well as frequently do not match the standards for halal dishes due to religious considerations. As a result, many

Corresponding author: hasan@uthm.edu.my

researchers are growing interested in fish gelatin as an alternative to mammals' gelatin. In Judaism, fish products with removable scales that can be removed without harming the skin are permitted, however, all fish are permitted in Islam [6]. The wasted part of the fish, particularly the skin can be used for gelatin extraction as it contains four times as much collagen as heads or bones [12]. Treatment that is favorable for fish collagen by acidic treatment as it has less crosslinking of covalent bonds that lead to gelatin type A [5]. Based on Al-Nimry et. al., the global output of gelatin from pig skin is regarded as the largest, accounting for 46% of the total production of gelatin, whereas fish production only accounts for 1.5% of total production.

Therefore, this study is focused on the extraction of gelatin from Salmon skin, as well as determining the effect of hydrochloric acid (HCl) on gelatin extraction in terms of gelatin yield, moisture, and possible functional group interactions in gelatin as well as salmon skin that will benefit various industries.

2. Materials and Methods

2.1 Materials

The raw material used in this study is salmon skins that were obtained from the fillet industry. The fish skins were refrigerated at -20℃. The storage limitation is within 2 months to produce better results. Frozen skins were thawed at room temperature before manually removing the attached flesh and scales.

2.2 Extraction of Gelatin

The fish skins were cleansed by running tap water to eliminate undesirable materials. The skins were cut into small pieces ($2\text{cm} \times 1\text{cm}$) in 50 grams. Then, the fish skins were immersed in different concentrations of hydrochloric acid (HCl) (0.05M, 0.1M, 0.5M, and 1.0M) (1:6 w/v) and times (6 hours, 18 hours, and 24 hours). After that, the skins were washed several times by running tap water before rinsing with distilled water to achieve pH 7. The final extraction was carried out using distilled water (1:3 w/v) at 50℃ for 4 hours in a water bath. Lastly, the solutions were filtered using Whatman filter paper No. 4, evaporated, and dried in an oven at 60℃ for 24 hours until brittle sheets were formed. The samples were weighed after each extraction process.

2.3 Yield of Gelatin

The percentage yield of gelatins was calculated based on the wet weight of the skins (Equation 1).

$$\%\ \text{Yield} = \frac{\text{Dry weight of gelatin (g)}}{\text{Wet weight of skins (g)}} \times 100 \quad \text{Eq. 1}$$

2.4 Moisture Content

The moisture content of the gelatins was calculated by using Equation 2.

$$\%\ \text{Moisture} = \frac{(W_1 - W_2)}{W_1} \times 100 \quad \text{Eq. 2}$$

2.5 Fourier Transform Infrared Spectroscopy (FTIR)

The possible functional group interactions in salmon skin and gelatin samples were analyzed using Perkin Elmer (USA) FTIR spectroscopy. All spectra were recorded at a resolution of 4cm^{-1} with a range between $4000 - 600\ \text{cm}^{-1}$. The spectra of both skin and gelatins were acquired at 32 scans.

3. Results and Discussion

The sample at 6 hours with various HCl concentrations (0.05M, 0.1M, 0.5M, and 1.0M) is used in this section.

3.1 Yield of Gelatin

Figure 1 shows the yield of gelatin produced at different HCl concentrations for 6 hours. Based on the figure, the chart shows a decrease in yield percentage from sample 0.05M to 0.1M then increased starting from the sample at 0.5M to 1.0M. The bar chart clearly showing the 1.0M sample (38.29%) produced the heaviest yield of gelatin compared to samples at 0.05M (13.67%), 0.1M (4.91%), and 0.5M (10.48%). Effective pre-treatment and extraction processes for gelatin influenced the yield of the gelatin. The higher concentration of HCl, the higher percentage of gelatin produced [9]. The low value of gelatin yield might be attributed to collagen loss during subsequent processing stages such as washing, pre-treatment, and extraction [18]. From the result obtained, gelatin extraction at 1.0M HCl for 24 hours is the most suitable parameter for producing a high yield of gelatin. Previous research obtained 74% of salmon skin gelatin yield by utilizing the procedure of mincing the skins and water extraction [12]. The yield from the previous research is double compared to the data obtained from this study. This may be influenced by the method utilized in extracting the gelatin.

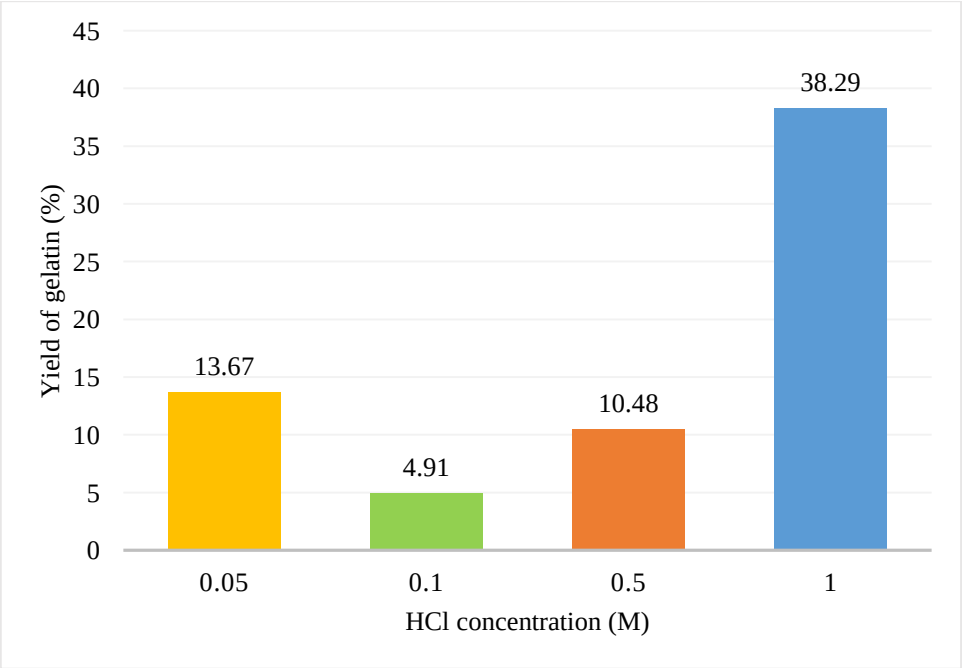

Figure 1: Yield of gelatin at different HCl concentrations for 6 hours

3.2 Moisture Content

Figure 2 shows the percentage of moisture content in extracted gelatin at various concentrations of HCl for 6 hours. The gelatin moisture increased from the sample at 0.05M to 0.1M and then decreased starting from the sample at 0.5M to 1.0M. The bar chart shows the sample at 0.1M (97.69%) obtained the highest percentage of moisture followed by the sample at 0.5M (95.33%), 1.0M (90.99%), and 0.05M (90.95%). Based on the data obtained, this salmon skin gelatin is not suitable to be utilized in industries as the Food and Agriculture Organization (FAO) has set the requirements for the moisture of the gelatin to not more than 18% [17]. The moisture content was most probably affected by the contribution of chemical residuals after treatment or interaction with other compounds [8].

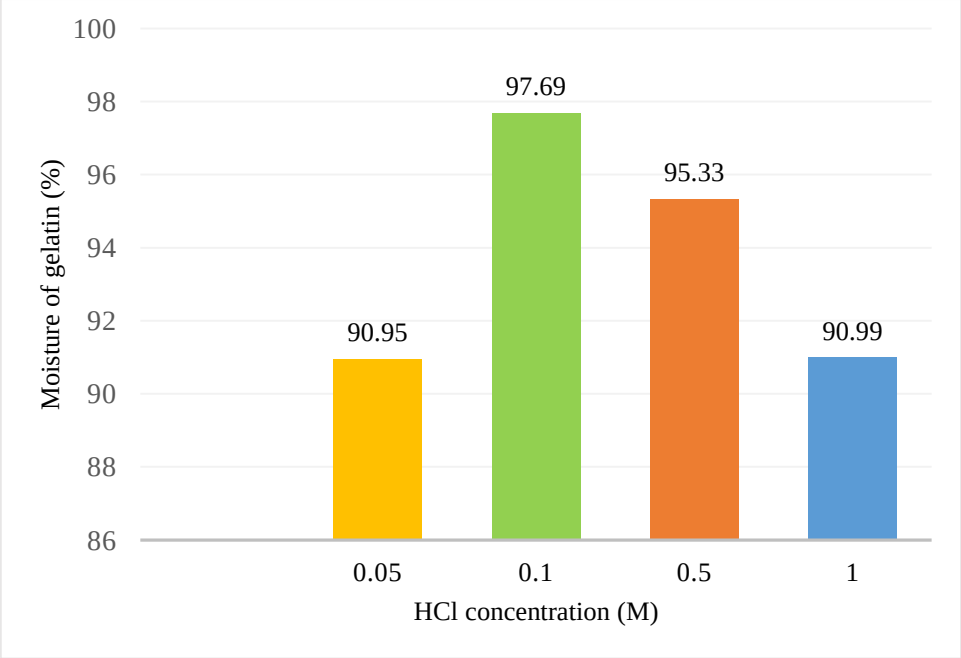

Figure 2: The moisture content in extracted salmon skin gelatin at 6 hours

3.3 Fourier Transform Infrared Spectroscopy (FTIR) Analysis

FTIR had been used on raw Salmon skin and extracted gelatin to determine the functional groups in them. All spectra were obtained at 32 scans in transmission mode with a resolution of 4 cm^{-1} and a range of 4000 - 600 cm^{-1}. Figure 3 shows the FTIR spectrum for raw salmon skin. It shows that raw salmon skin has high intensity of Amide A and Amide I with frequencies of 3278.49 cm^{-1} and 1637.03 cm^{-1} respectively. The peaks at a frequency of 2924.48 cm^{-1} and 1548.53 cm^{-1} indicate the low intensity of raw skins to Amide B and Amide II respectively.

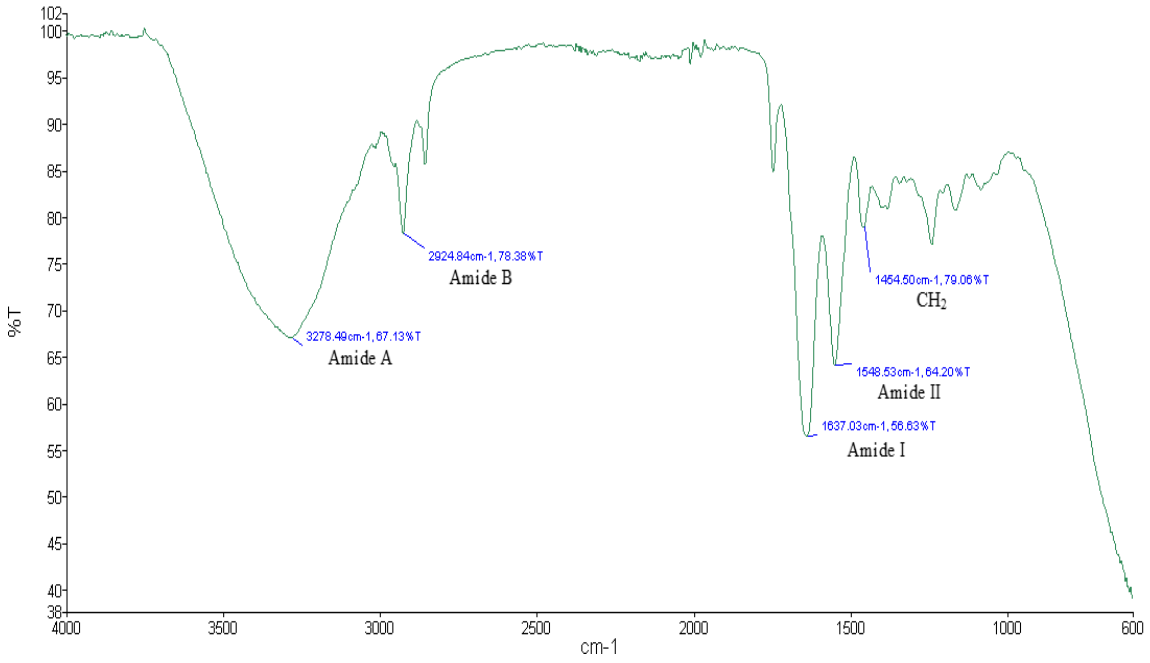

Figure 3: FTIR spectra of raw salmon skin

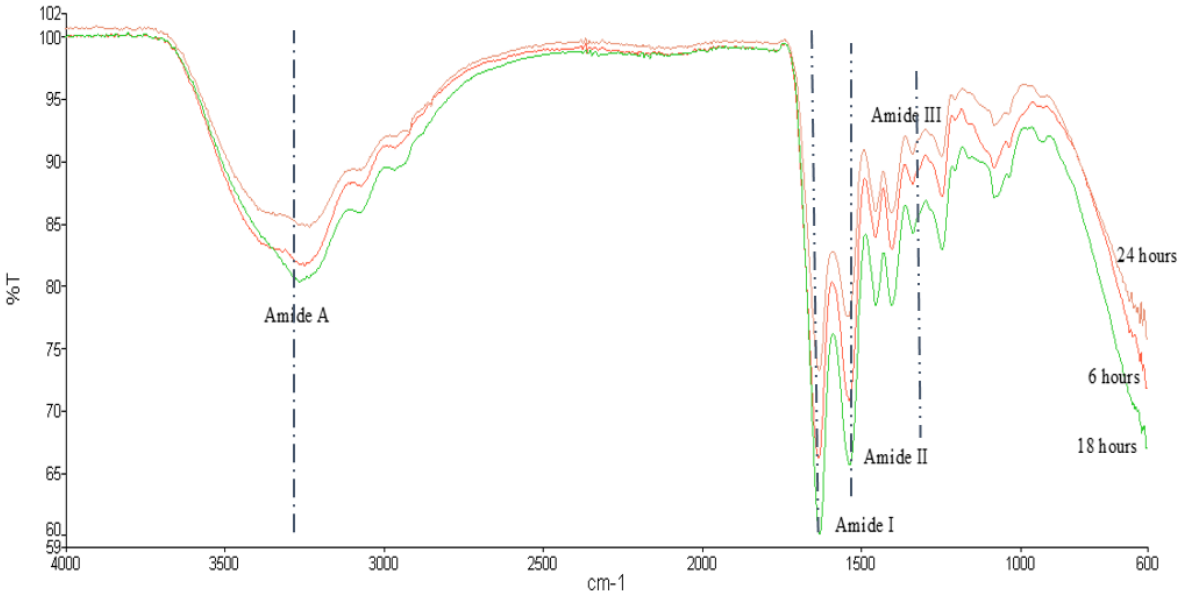

Figure 4: FTIR spectra on extracted gelatin (0.05M)

Table 1: FTIR spectra locations for extracted gelatin (0.05M)

Regions	Wavelengths (cm^{-1})		
	6 hours	**18 hours**	**24 hours**
Amide A	3246.24	3264.34	3234.02
Amide I	1631.29	1629.59	1630.46
Amide II	1534.90	1534.94	1536.50
Amide III	1243.40	1335.35	1336.69

Figure 4 shows the FTIR spectra on extracted gelatin at 0.05M while table 1 shows the summary of the wavelength value of each region on the graph. The graph shows that gelatin extraction at 18 hours has the highest intensity of Amide A, Amide I, and Amide II compared to gelatin extraction at 6 hours and 24 hours. Those three different times of extracted gelation show similar intensity for Amide III. The Amide B band that has not been detected by the FTIR might be attributable to collagen loss during the following processing stages such as washing, pre-treatment, and extraction resulting in the FTIR being unable to identify the low collagen value in the gelatin sheets. Amide A in these FTIR spectra was recognized as a hydrogen-bonded hydroxyl group (O-H) [14]. The lower frequency of Amide A indicates an increase in hydrogen bonding between collagen molecules [14]. The presence of collagen is indicated by the presence of an Amide B band [18]. Amide B band also shows the asymmetrical and symmetrical CH_2 stretch [4]. Amide I ($1600cm^{-1}$ - $1700cm^{-1}$) is classified as a secondary structure of the protein [14] and represents the stretching vibrations of protein C=O groups [4]. The peaks of Amide II correspond to the NH bending vibration coupled with CN stretching [4]. Amide III demonstrated the presence of helical structure [14] as well as correlates to the NH bending associated with CN stretching [4]. Furthermore, Amide III was clearly shown in fish skin gelatin whereas it was significantly low in bovine and porcine gelatin [3].

4. Conclusion

In conclusion, this study is focused on the extraction of gelatin from Salmon skin as well as determining the effect of hydrochloric acid (HCl) on gelatin extraction. The highest yield of gelatin is at an HCl concentration of 1.0M for 6 hours (38.29%). The results of this study indicate that 1.0M with 6 hours of pre-treatment process is the most suitable for producing a high yield of gelatin from salmon skin. The moisture of the gelatin decreased as the pre-treatment (HCl concentration and times)

increased. The properties of the gelatin are determined by Fourier Transform Infrared Spectroscopy (FTIR). FTIR spectra for extracted gelatin at various HCl concentrations and times showed a similar pattern. The raw salmon skin contains collagen (Amide B) which makes it a suitable material for gelatin extraction. The information in this study is useful and could lead to further studies in producing gelatin from salmon skin that will benefit various industries.

Acknowledgement

The authors acknowledge with gratitude the support provided by Universiti Tun Hussein Onn Malaysia to conduct this study. The authors would also like to thank the Faculty of Mechanical and Manufacturing Engineering, Universiti Tun Hussein Onn Malaysia for its support.

References

[1] Abd Elgadir, M., Mirghani, M. E., & Adam, A. (2013). Fish gelatin and its applications in selected pharmaceutical aspects as alternative source to pork gelatin. *J. Food Agric. Environ*, *11*, 73-79.\

[2] Al-Nimry, S., Dayah, A. A., Hasan, I., & Daghmash, R. (2021). Cosmetic, biomedical and pharmaceutical applications of fish gelatin/hydrolysates. *Marine Drugs*, *19*(3), 145.

[3] Al-Saidi, G. S., Al-Alawi, A., Rahman, M. S., & Guizani, N. (2012). Fourier transform infrared (FTIR) spectroscopic study of extracted gelatin from shaari (*Lithrinus microdon)* skin: effects of extraction conditions.

[4] Alves, A. L., Marques, A. L., Martins, E., Silva, T. H., & Reis, R. L. (2017). Cosmetic potential of marine fish skin collagen. *Cosmetics*, *4*(4), 39.

[5] Boran, G., & Regenstein, J. M. (2009). Optimization of gelatin extraction from silver carp skin. *Journal of food science*, *74*(8), E432-E441.

[6] Choi, S. S., & Regenstein, J. M. (2000). Physicochemical and sensory characteristics of fish gelatin. *Journal of Food Science*, *65*(2), 194-199

[7] Deshmukh, K., Basheer Ahamed, M., Deshmukh, R. R., Khadheer Pasha, S. K., Bhagat, P. R., & Chidambaram, K. (2017). *Biopolymer Composites With High Dielectric Performance: Interface Engineering. Biopolymer Composites in Electronics*, *27–128.* doi:10.1016/b978-0-12-809261-3.00003-6

[8] Ismail, N., & Abdullah, H. Z. (2016). Effects of hydrochloric acid concentration on the properties of black tilapia fish skin gelatin. In *Materials Science Forum* (Vol. 840, pp. 165-169). Trans Tech Publications Ltd.

[9] Ismail, N., & Abdullah, H. Z. (2019). The extraction of gelatin from black tilapia fish skins with different acid concentration. In *Journal of Physics: Conference Series* (Vol. 1150, No. 1, p. 012041). IOP Publishing.

[10] Karim, A. A., & Bhat, R. (2009). Fish gelatin: properties, challenges, and prospects as an alternative to mammalian gelatins. *Food hydrocolloids*, *23*(3), 563-576.

[11] Keenan, T. R. (2012). *Gelatin. Polymer Science: A Comprehensive Reference, 237–247.* doi:10.1016/b978-0-444-53349-4.00265-x

[12] Kołodziejska, I., Skierka, E., Sadowska, M., Kołodziejski, W., & Niecikowska, C. (2008). Effect of extracting time and temperature on yield of gelatin from different fish offal. *Food Chemistry*, *107*(2), 700-706.

[13] León-López, A., Morales-Peñaloza, A., Martínez-Juárez, V. M., Vargas-Torres, A., Zeugolis, D. I., & Aguirre-Álvarez, G. (2019). Hydrolyzed collagen—Sources and applications. *Molecules*, *24*(22), 4031.

[14] Liu, H., Zhao, L., Guo, S., Xia, Y., & Zhou, P. (2014). Modification of fish skin collagen film and absorption property of tannic acid. *Journal of Food Science and Technology*, *51*(6), 1102-1109.

[15] Narayanaswamy, R., Kanagesan, S., Pandurangan, A., & Padmanabhan, P. (2016). *Basics to different imaging techniques, different nanobiomaterials for image enhancement. Nanobiomaterials in Medical Imaging, 101–129.* doi:10.1016/b978-0-323-41736-5.00004-2

[16] Schrieber, R.; Gareis, H. *Gelatine Handbook: Theory and Industrial Practice*, 1st ed.; John Wiley & Sons: Eberbach, Germany, 2007; p. 347.

[17] Setyawaty, R., & Triliandari, M. (2018). Gelatin Production from Skin of Chicken Leg using A Variety of Naoh Concentration. *Jurnal Ilmu dan Teknologi Hasil Ternak (JITEK)*, *13*(2), 126-132.

[18] Sockalingam, K., & Abdullah, H. Z. (2015). Extraction and characterization of gelatin biopolymer from black tilapia (Oreochromis mossambicus) scales. In *AIP conference proceedings* (Vol. 1669, No. 1, p. 020053). AIP Publishing LLC.

[19] Vázquez, J. A., Hermida-Merino, C., Hermida-Merino, D., Piñeiro, M. M., Johansen, J., Sotelo, C. G., Pérez-Martín, R.I. & Valcarcel, J. (2021). Characterization of gelatin and hydrolysates from valorization of farmed salmon skin by-products. *Polymers*, *13*(16), 2828