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Extraction of collagen from marine sources of sardine & emperor fish scale wastes

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Abstract
Collagen from fish scales serves to lower the risk of consuming collagen from other sources while also reducing land pollution. The scales of the sardine and emperor fish breeds were coughed up heavily and had more number of scales. The pretreatment, hydrolysis, and extraction procedure ratios applied to the scales of two different fish breeds varied according to the water level and temperature. The microstructure, elements, and functional groups were analyzed using SEM and FTIR. The results of the SEM and FTIR analysis demonstrated the most suitable collagen powder extraction ratio. The purest collagen was produced using 5 grams of emperor fish scales, 0.1 N NaOH for 3 days of pretreatment, 0.5 M HCl for 3 days of hydrolyzation, and 50 ml of double-distilled water utilized at 80°C. Additionally, this study demonstrated that collagen may be obtained by the drying process at room temperature. Comparing this procedure to freeze drying, it is less expensive. Moreover, this isolation was done by without using NaCl.

Keywords: Emperor fish scale, sardine fish scale, hydro extraction, collagen, marine waste

1. Introduction
Every day, marine wastes, particularly fish wastes, were dumped into dustbins and sea areas. It is high in collagen-forming proteins. The majority of fish that live in the ocean contain type 1 collagen. Type 1 collagen was found in every living organism. Collagen extraction can take place via a large amount of fish waste. Therefore, the collagen extraction process used marine sources such as the scales of sardine and emperor fish. Because compared to other fish breeds, it has a higher supply of fish scales and is available throughout the year.

1.1 Fish Wastes
Fish processing locations such as fish markets and industries threw out fish bones, liver, head, tail, fins, scales, and skins. However, on chicken farms, fish bones, livers, heads, tails, skins, and fins were used as chicken feed. Fish scale wastes are not as concentrated as other fish component wastes.

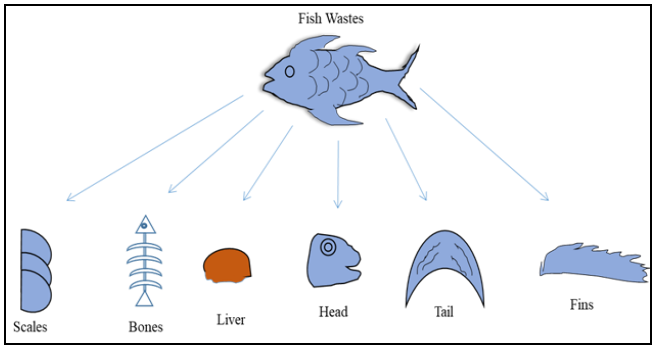


Fig 1: Parts of Fish Wastes

All fish waste products contain organic materials. Additionally, the scale has less collagen than other fish sections. However, the fish processing only throws out the scales entirely. Therefore, the best strategy to use fish scales and manage bio-wastes is to extract collagen from them.

1.2 Collagen

Collagen is a fibrous protein that is present in all multicellular animals and is the most common protein in vertebrates [1]. In developed tissue, it serves as a structural component, and in maturing tissue, it provides guidance [2]. The excellent properties of collagen biomaterials such as their hydrophilicity, biodegradability, biocompatibility, low immunogenicity, and ease of processing have raised a lot of interest in biological fields [3]. From both plant [4] and animal [5] sources, the collagen was isolated it.

2. Material and Methods

The following steps were carried out for the collagen extraction process.

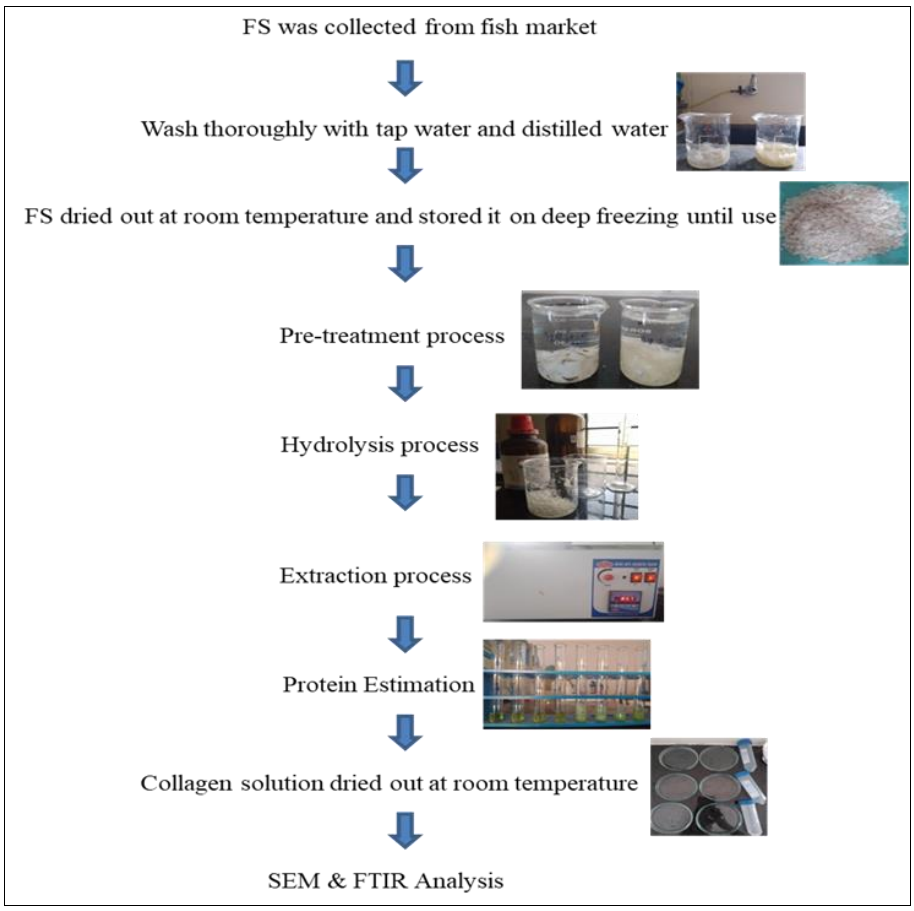


Fig 2: Flow chart of the hydro extraction process for collagen extraction from emperor and sardine fish scales

2.1 Materials and Equipments

Fish scales served as the primary study material. Scales from sardines and emperor fish were gathered from the fish market and distilled water (both single and double), hydrochloric acid (HCl), and sodium hydroxide (NaOH). For the protein estimation, it employed bovine serum albumin, sodium carbonate, cupric sulphate, potassium sodium tartrate, and folin. Collagen was extracted using a Water Bath Shaker instrument. The protein was estimated using a UV spectrophotometer, the structure and component analysis was done with a scanning electron microscope (SEM), and the functional groups were found using Fourier Transform Infrared Spectroscopy (FTIR).

2.2 Pretreatment Process

The Sardine and the Emperor Fish scales were gathered from the fish market and removed every last particle of leftover skin and bone. Fish scales were collected and properly cleaned with tap water and being cleaned three times with distilled water.

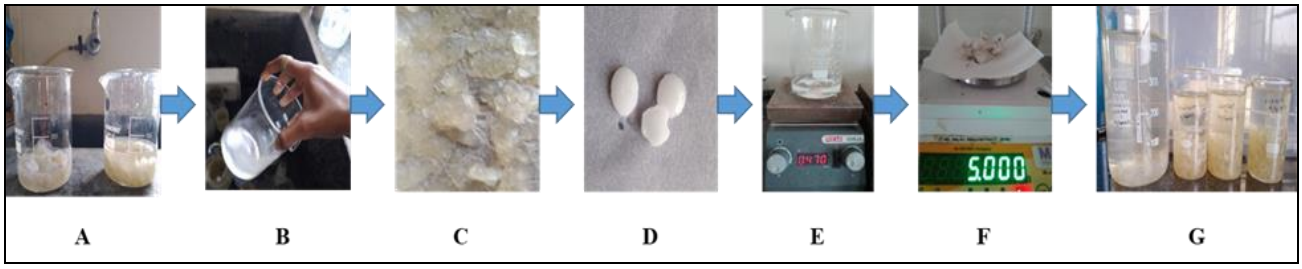


Fig 3: A-washed FS with tap water, B-Washed FS with distilled water, C-FS were dried out at room temperature, D-NaOH pellets for preparing the solution, E-NaOH solution, F-Fish scales at grams, G-Fish Scales in NaOH Solution for pre-treatment.

Before being used, a cleaned fish scale was allowed to air dry at room temperature and then frozen. For three days, 0.1 and

0.5 N NaOH solutions were applied to 3 and 5 grams of sardine and emperor fish scales, respectively.

2.3 Hydrolysis Process: Three full washes with distilled water were used to eliminate all contaminants and mineral compounds from the fish scales that had been pre-treated. Following pretreatment, 0.5 M HCl was applied to the fish scales for three and four days, respectively.
It is used to easily extract collagen from fish scales by eliminating non-collagenous components. After that, distilled water was used three times to properly wash the hydrolyzed fish scales.

Fig 4: Hydrolysis-A-Washed FS with distilled water after pretreatment, B-Prepare 0.5M of HCl solution, C-FS treated with HCl solution for three days, D-Washed FS with distilled water after hydrolysis.

2.4 Extraction of Collagen
Hydro-Extraction process was used for extraction of collagen from fish scales. According to [6] author mentioned, in comparison to the extraction method of acid and pepsin, it involves less chemicals. Compared to the pepsin extraction process, it is relatively inexpensive.

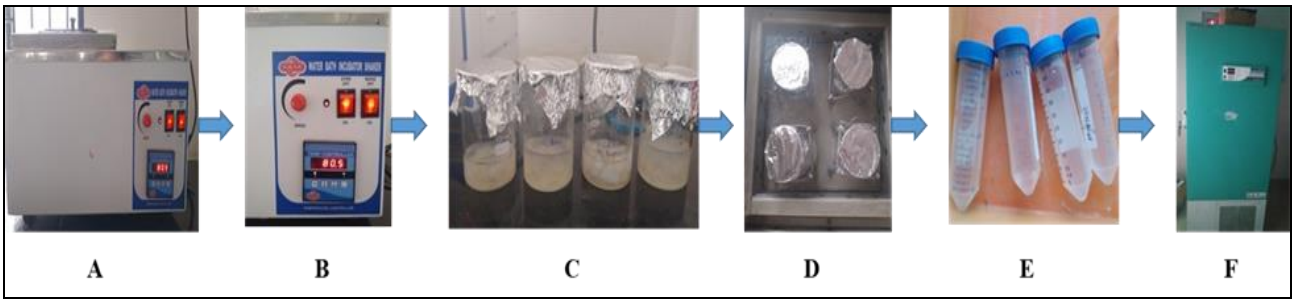


Fig 5: Extraction-A-Water Bath Shaker, B-Set a temperature and rpm with stirring, C-take a FS with double distilled water, D-fit the samples to the water bath, E-filtered the extracted collagen solution, F-the collagen solution was stored in deep freezing at-18°C.

For the extraction, fish scales were obtained at levels 40, 50, and 60 using double-distilled water. Collagen solution is extracted at temperatures of 40, 60, and 80°C using a water bath shaker.

2.5 Collagen Powder: The majority of the authors [7] dried out their collagen solutions using the freeze-drying approach. Rather than using the freeze-dry approach, the drying process in this investigation was done at room temperature.
Each extracted collagen solution was dried off independently, and the powder's texture and amount were examined. In order to obtain collagen powder, the isolated collagen solution was dried at room temperature. The collagen powder was measured using a weight balance apparatus.

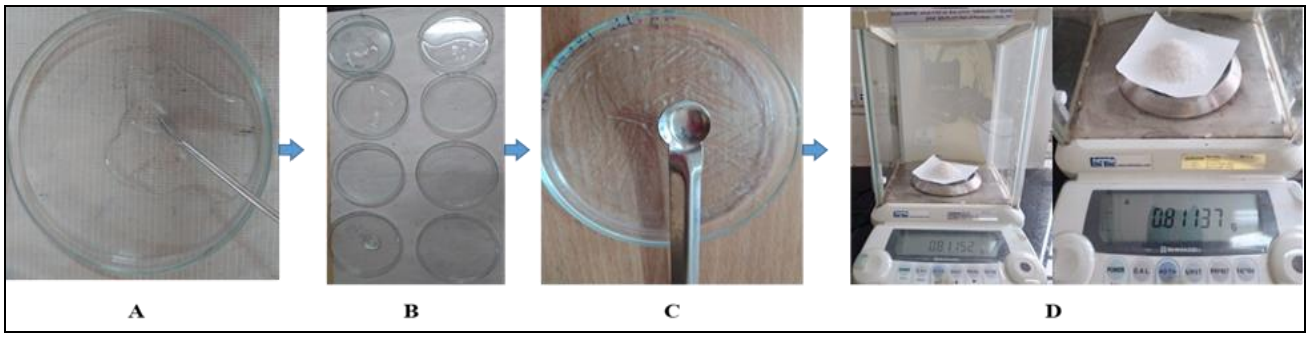


Fig 6: A & B-collagen solution was dried out separately at room temperature for 2-3 days, C-scratch the dried solution to collect the powder, D-measure the weight of the collagen powder

3. Results and Discussion
3.1 Collagen Estimation: The Lowry assay method was used to estimate the amount of protein. Bovine serum albumin was the stock solution used in this procedure. The extracted collagen solution's protein content was determined using a UV Spectrophotometer.

Table 1: Determination of Collagen powder in different extraction ratios

S. No	Fish Scales	Grams	Pre-treatment		Hydrolysis		Extraction				Result P (Powder)
			NaOH	Days	HCl	Days	Hrs	Rpm	Stirring	Temperature	
1	Sardine	5g	0.1N	3	0.5M	4	2	M	On	40°c	-
2	Emperor	5g	0.1N	3	0.5M	4	2	M	On	40°c	-
3	Sardine	5g	0.5N	3	0.5M	4	2	M	On	40°c	-
4	Emperor	5g	0.5N	3	0.5M	4	2	M	On	40°c	P
5	Sardine	5g	0.1N	3	0.5M	3	2	M	On	60°c	P
6	Emperor	5g	0.1N	3	0.5M	3	2	M	On	60°c	P
7	Sardine	3g	0.1N	3	0.5M	3	2	M	On	80°c	P
8	Emperor	3g	0.1N	3	0.5M	3	2	M	On	80°c	-
9	Sardine	5g	0.1N	3	0.5M	3	2	M	On	80°c	P
10	Emperor	5g	0.1N	3	0.5M	3	2	M	On	80°c	P

Table 1-Changes were made to the pre-treatment normality, hydrolysis process days, and extraction temperature in order to extract the collagen from three and five grams of sardine and emperor fish scales, respectively. From 5 grams of pre-treated FS in 0.1N NaOH at 40 °C, collagen powder was not obtained. However, the small amount of collagen powder came from emperor scales that had been pre-treated with 0.5N of NaOH. Five grams of both fish scales were powdered at 60 °C, which is a temperature higher than that of 40 °C extraction. Furthermore, 3 grams of the collagen powder from the emperor scale extraction at 80 °C were not obtained. Similar to sardine scales, only 5 grams of emperor scale collagen powder at 80°C was obtained for the same amount of collagen. Ultimately, 5 grams of both fish scales that had been pre-treated with 0.1N NaOH, hydrolyzed for 0.5M, and extracted at 80 °C produced the maximum collagen yields.

Table 2: Determination of collagen quantity level in different water level of extraction

S. No	Fish Scales	Grams	Pre-treatment		Hydrolysis		Extraction				Quantity of Collagen (grams)
			NaOH	Days	HCl	Days	Hrs	DDW (ml)	Stirring	Temperature	
1	Sardine	5g	0.1N	3	0.5M	3	2	40	On	80°c	0.34
2	Emperor	5g	0.1N	3	0.5M	3	2	40	On	80°c	0.73
3	Sardine	5g	0.1N	3	0.5M	3	2	50	On	80°c	0.42
4	Emperor	5g	0.1N	3	0.5M	3	2	50	On	80°c	0.81
5	Sardine	5g	0.1N	3	0.5M	3	2	60	On	80°c	0.58
6	Emperor	5g	0.1N	3	0.5M	3	2	60	On	80°c	0.96

Table 2, The most collagen could be obtained from 5g of both Emperor and Sardine fish scales when 0.1N NaOH was used as a pretreatment and 0.5 M HCl was employed as a hydrolysis agent at a temperature of 80°C, according to earlier study and analysis. 40, 50, and 60 ml of distilled water were used for the collagen extraction. Consumption of collagen powder derived from Emperor Scale was higher than that of Sardine scale extract. It was also found that the amount of powder increased in proportion to the water level. However, at the same time, the texture and thickness changed according on the water level. Compared to the powder in the 50 and 60 ml solutions, the 40 ml collagen powder was much tougher. The collagen powder in the 60 ml solution had a much finer and lighter texture than the powder in the 40 & 50 ml solutions. The ideal texture for collagen powder was achieved with 50 ml of extracted solutions.

3.2 SEM Analysis

SEM analysis was carried out for analyzing the microstructure and morphology of raw fish scales and extracted collagen powder.

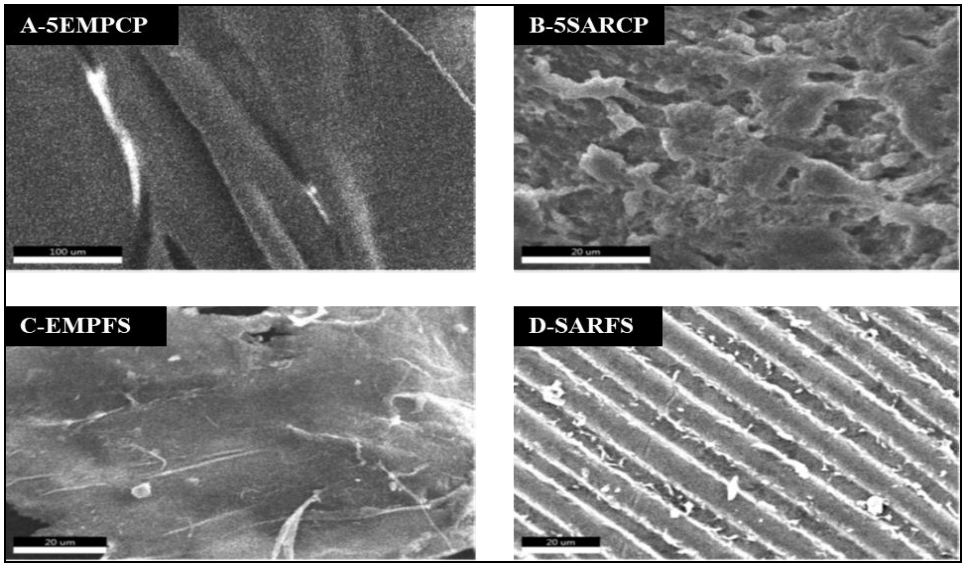
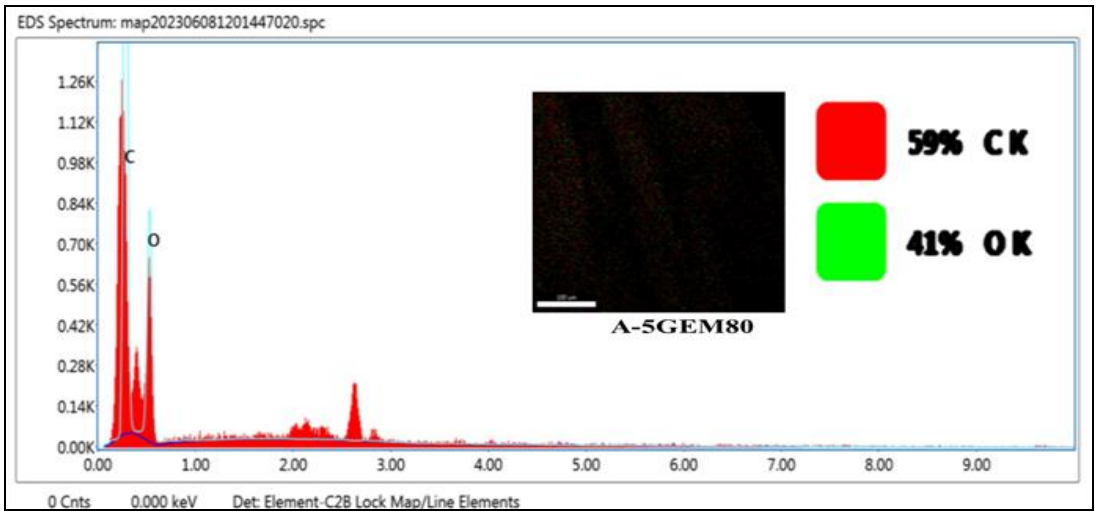


Fig 7: SEM micrographs depicting the surface morphology of A-Emperor collagen powder (5EMPCP), B-Sardine collagen powder (5SARCP), C-Emperor fish scale (EMPFS), D-Sardine fish scale (SARFS)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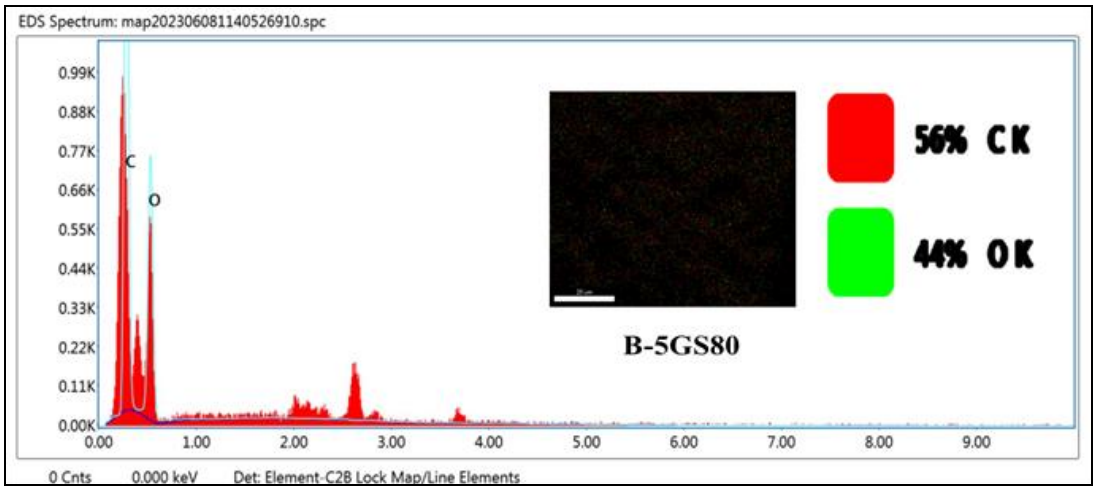
1.12K
0.98K
0.84K
0.70K
0.56K
0.42K
0.28K
0.14K
0.00K
C
O
0.00 1.00 2.00 3.00 4.00 5.00 6.00 7.00 8.00 9.00
0 Cnts 0.000 keV Det: Element-C2B Lock Map/Line Elements
59% CK
41% OK
A-5GEM80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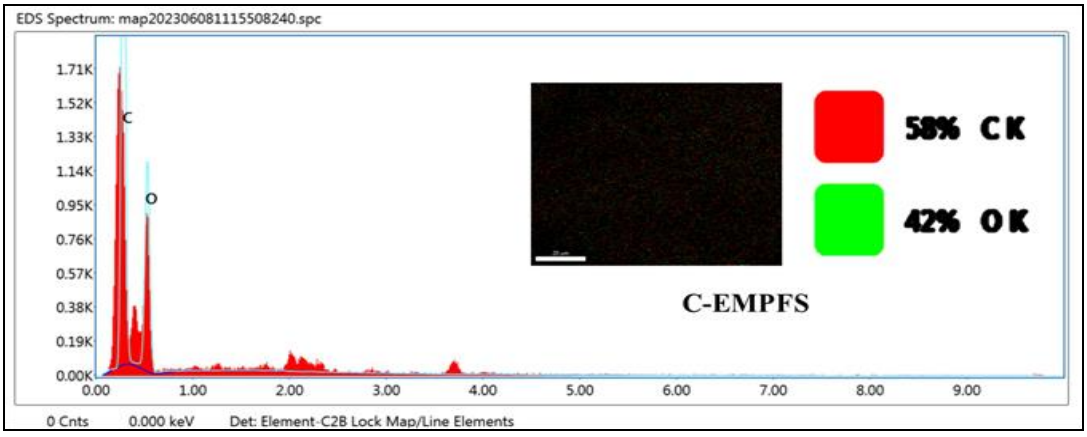
0.88K
0.77K
0.66K
0.55K
0.44K
0.33K
0.22K
0.11K
0.00K
C
O
0.00 1.00 2.00 3.00 4.00 5.00 6.00 7.00 8.00 9.00
0 Cnts 0.000 keV Det: Element-C2B Lock Map/Line Elements
56% CK
44% OK
B-5GS80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0.57K
0.38K
0.19K
0.00K
C
O
0.00 1.00 2.00 3.00 4.00 5.00 6.00 7.00 8.00 9.00
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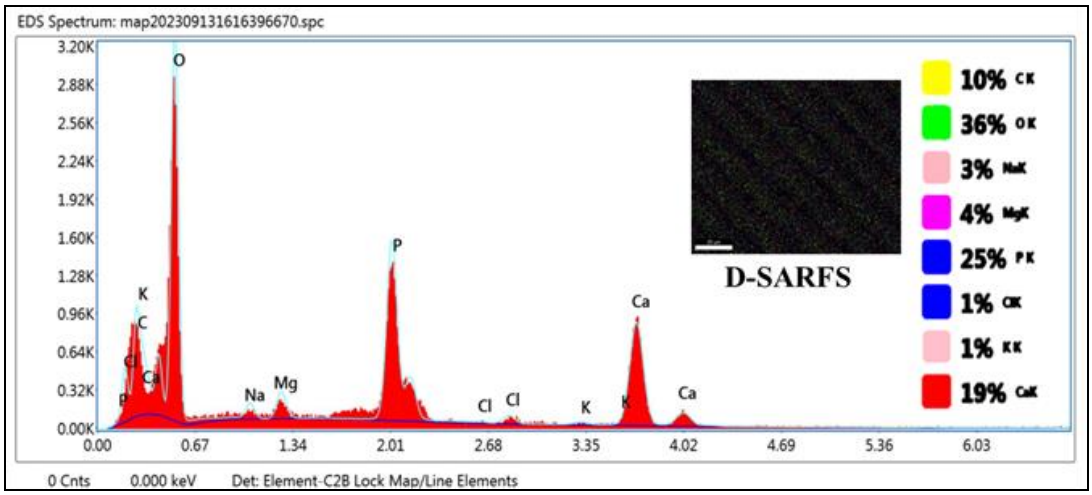
58% CK
42% OK
C-EMPFS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2.56K
2.24K
1.92K
1.60K
1.28K
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0.64K
0.32K
0.00K
O
K
C
Cl
Ca
P
Na
Mg
Cl
Cl
K
K
Ca
Ca
0.00 0.67 1.34 2.01 2.68 3.35 4.02 4.69 5.36 6.03
0 Cnts 0.000 keV Det: Element-C2B Lock Map/Line Elements
10% CK
36% OK
3% NaK
4% MgK
25% PK
1% ClK
1% KK
19% CaK
D-SARFS

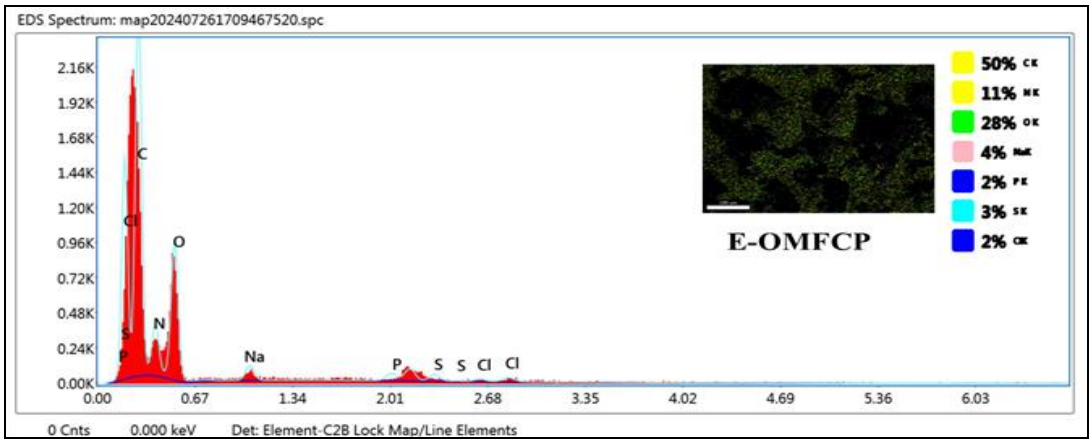

Fig 8. EDS Spectrum mapping of A-Emperor Collagen powder at 80°C extracted (5GEM80), B-Sardine Collagen powder at 80°C extracted (5GS80), C-Original Emperor fish scale (EMPFS), D-Original Sardine fish scale (SARFS), E-Original Marine Fish Collagen Powder

Figure 7 A was showed the fine structure of Emperor Collagen powder than Sardine Collagen powder (Fig; 7-B). Also Fig; 7-C showed the fine structure of emperor fish scales than sardine fish scale (Fig; 7-D). A raw fish scale of Emperor (Fig; 8-C) has a high value of carbon and oxygen components and also has low level of impurities than sardine fish scale (Fig; 8-D). The sardine fish scale (Fig; 8-D) has multi components than the emperor fish scales (Fig; 8-C). Extracted Collagen powder from both fish scales of emperor and sardine has a high amount of carbon and oxygen components. After extraction, both scales of Collagen powder's oxygen and carbon level were increased than the raw fish scales.

3.3 FTIR Analysis

The signals were collected using 25 scans over the range of 4000-400 cm^{-1} at a resolution of 4 cm^{-1}

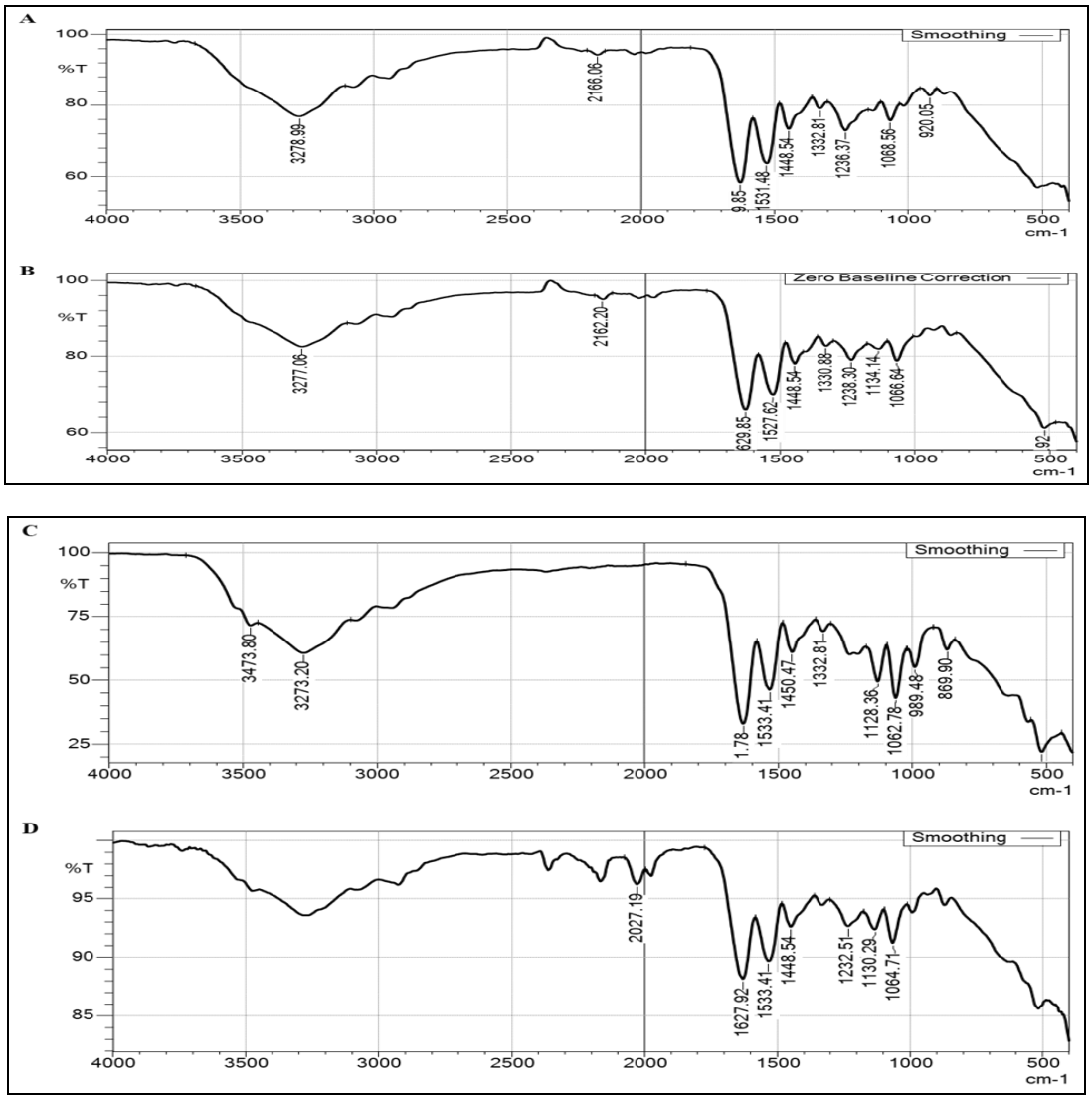

Fig 9: FTIR spectroscopy of Emperor FS Collagen powder at 60°C extraction (A-5E60), Sardine FS Collagen powder at 60°C extraction (B-5S60), Emperor FS Collagen powder at 80°C extraction (C-5E80), Sardine FS Collagen powder at 80°C extraction D-5S80

Table 3: FTIR Absorbency of 5E60

	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	920.05	82.76	2.10	956.69	894.97	993.748	59.340
2	1068.56	75.72	5.06	1103.28	1033.85	1504.410	170.303
3	1236.37	72.89	7.14	1303.88	1151.50	3518.302	456.298
4	1332.81	79.10	2.59	1361.74	1303.88	1131.875	72.244
5	1448.54	73.34	7.76	1483.26	1361.74	2701.678	450.063
6	1531.48	63.71	14.87	1581.63	1483.26	2914.007	803.463
7	1629.85	58.32	22.25	1815.02	1581.63	4304.309	1124.301
8	2166.06	94.23	1.18	2204.64	2137.13	349.101	40.430
9	3278.99	76.91	12.27	3672.47	3107.32	8642.098	3851.169

Table 4: FTIR Absorbency of 5S60

	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	516.92	61.17	4.15	844.82	476.42	9922.657	518.953
2	1066.64	78.79	5.79	1101.35	1006.84	1685.782	244.499
3	1134.14	81.94	1.86	1176.58	1101.35	1295.563	73.437
4	1238.30	78.96	4.77	1301.95	1176.58	2292.650	254.192
5	1330.88	82.69	2.00	1359.82	1301.95	943.779	58.013
6	1448.54	78.02	4.46	1481.33	1413.82	1329.359	144.265
7	1527.62	69.88	12.33	1581.63	1481.33	2464.783	668.980
8	1629.85	65.95	18.83	1772.58	1581.63	3270.362	1158.218
9	2162.20	95.00	1.37	2189.21	2123.63	279.064	44.066
10	3277.06	82.53	9.20	3670.54	3107.32	6409.668	2847.516

Table 5: FTIR Absorbency of 5E80

	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	514.99	22.08	10.76	555.50	441.70	8265.305	541.451
2	869.90	62.11	5.84	921.97	840.96	2689.762	148.899
3	989.48	55.28	9.95	1020.34	921.97	3549.296	283.978
4	1062.78	43.15	20.25	1095.57	1020.34	3478.315	718.683
5	1128.36	49.62	14.15	1172.72	1095.57	3298.074	499.096
6	1332.81	69.41	3.83	1361.74	1305.81	1602.884	107.419
7	1450.47	61.16	11.97	1485.19	1361.74	3955.492	674.202
8	1533.41	46.41	22.72	1581.63	1485.19	4146.980	1169.990
9	1631.78	33.13	38.07	1847.81	1581.63	6912.687	1752.218
10	3273.20	60.82	12.50	3446.79	3099.61	11538.185	2274.821
11	3473.80	71.71	3.68	3714.90	3446.79	3542.621	-224.929

Table 6: FTIR Absorbency of 5S80

	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	1064.71	91.22	3.05	1097.50	1010.70	608.591	115.565
2	1130.29	92.38	1.65	1174.65	1097.50	524.901	62.555
3	1232.51	92.66	1.71	1301.95	1174.65	811.939	101.977
4	1448.54	92.61	2.17	1483.26	1359.82	753.649	127.840
5	1533.41	89.69	4.30	1581.63	1483.26	826.216	235.605
6	1627.92	88.16	6.71	1772.58	1581.63	1128.123	441.103
7	2027.19	96.23	1.68	2075.41	1996.32	222.525	65.749

Collagen from sardine and emperor fish scales that were extracted at 60 and 80°C temperatures was subjected to FTIR analysis in order to determine and characterize the different functional groups in the collagen. Analysis was done on the secondary structure of collagens isolated from fish scales. Similar tendencies were observed in the spectrum by comparable bands of type 1 collagen with literature. This comprises the principal absorption band of amides A, B, I, II, and III. Amide A bands for Fig-9 and Tables-3, 4, 5 & 6 collagen were observed at 3278, 3277, 3473 and 3490 cm^{-1} respectively [7]. Amide B bands for collagen were observed at 2920, 2920, 2915 and 2910 cm^{-1} respectively. The asymmetric stretch of the CH_2 stretching vibration is associated with the Amide I band. The amide A and amide B wave numbers and amplitude variations seen in the collagen sources suggested that the secondary structure of collagen might differ, particularly between the collagen in Figs. A, B and C, D.

The amide I peak is linked to C=O stretching vibrations, hydrogen bond coupling with COO-, or stretching (1600-1700 cm^{-1}) [8]. Amide I bands for Fig; 9-A, B C & D collagen were observed at 1629, 1629, 1631 and 1627 cm^{-1} respectively. The N-H group's involvement in hydrogen bonding is indicated by the amide II band's interaction with N-H bending vibration paired with C-N stretch [9]. Amide II bands for Fig-9 and Tables-3, 4, 5 & 6 collagen were observed at 1531, 1527, 1533 and 1533 cm^{-1} respectively. Amide III belongs to C-O stretching, N-H bend, and C-N stretching vibrations. Amide III peak and intensity is a key collagen property [10]. Amide I bands for Fig; 8-A, B C & D collagen were observed at 1236, 1238, 1237 and 1232 cm^{-1} respectively.

4. Conclusion

The challenge of how to transform such garbage into a usable

item is crucial. Currently, research is being done on fish scale waste collagen as a possible biological resource to substitute collagen from animals. The findings of this study indicate that it is possible to make collagen powder at room temperature using fish scale trash. The optimal collagen extraction ratio, as determined by SEM and FTIR results, is 5 grams of emperor fish scales that have been pretreated with 0.1N NaOH, hydrolyzed with 0.5M HCl, and extracted with 50 ml of water at 80°C. At 80°C, every FTIR absorbency level was at an acceptable level. The collagen powder from the emperor fish scale was more pure than that from the sardine scale. A significant, cost-effective, and abundant source of fish collagen is provided by marine resources, particularly fish waste sources, which can be used for a variety of purposes.

5. Acknowledgement

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6. Declaration of interests

The authors didn't receive support from any organization for the submitted work.

7. References

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