International Journal for Multidisciplinary Research (IJFMR)
E-ISSN: 2582-2160 ● Website: www.ijfmr.com ● Email: editor@ijfmr.com

Gelatin Extraction and Characterization from Fish Scale Wastes

K. Sathish Kumar[1], H. Ann Suj[2], S. Sanathani[3]

[1,2,3]Center of Advanced Study in Marine Biology, Annamalai University, Parangipettai, Tamil Nadu – 608 502, India

ABSTRACT

Waste materials are a major environmental concern in fisheries and aquaculture industries worldwide. This research investigated the use of bacteria to aid in extracting gelatin from fish scales, with a focus on maximising yield and analysing the physicochemical properties of the produced gelatin. Five bacterial strains capable of breaking down gelatin were isolated from the scales of many fish species, including the species *Aneurinibacillus aneurinilyticus* and *Enterobacter cloacae.* Gelatin yields were found to vary between 9.72% and 13.2%, accompanied by protein contents ranging from 7% to 12.06%, confirming the effective enzymatic degradation of collagen by these bacteria. The extracted gelatin exhibited slightly acidic pH values and retained its structural characteristics, as confirmed by FTIR spectroscopy, which identified key amide functional groups. This microbially driven extraction approach minimises the use of harsh chemicals, offering a more sustainable method to convert fish scale waste into valuable gelatin. The findings support the potential development of eco-friendly gelatin production methods with applications across industrial sectors. Future studies should aim to improve bacterial strain performance and scale-up processes for commercial feasibility.

Keywords: Fish scale, gelatin, *Aneurinibacillus aneurinilyticus*, *Enterobacter cloacae*, PCR amplification, FTIR analysis.

INTRODUCTION

Fish are a primary source of high-quality protein, supplying 15–20 % protein in many diets worldwide [1][2] (Fagbenro et al. 2005; Usman et al. 2022). The rapid growth of global fisheries—approximately 179 Mt of catch in 2018—has intensified processing activities, yet the industry generates a staggering amount of waste. An estimated 7.3 Mt of skins, bones, fins, and other by-products are discarded each year, and up to 85 % of the harvested biomass can become low-value waste [3][4] (Kelleher 2005; Arvanitoyannis et al. 2008). Only about 78 % of the total catch is directed toward human consumption, while the remaining 21 % supports non-food uses such as bait, animal feed, or fertiliser [5] (Vannuccini 2004).

Among these residues, fish scales are particularly noteworthy. Scales account for roughly 3 % of the total fish weight and are composed of a highly ordered composite of type I collagen and calcium-deficient hydroxyapatite [6] (He et al. 2022). Structurally, 41–45 % of a scale is organic material—including collagen, proteins, lecithin, vitamins, and trace minerals—whereas 38–46 % is inorganic, mainly hydroxyapatite and calcium phosphate, with additional trace elements such as calcium, magnesium, iron, and zinc [6] (He et al. 2022). These components give scales a unique combination of flexibility, strength,

and hardness that resembles human bone, teeth, and mineralised tendons, making them attractive for biomedical and functional material applications [7] (Shyni et al. 2013).

Gelatin, a partially hydrolysed form of collagen, is a biodegradable polymer with a broad spectrum of uses in food, pharmaceuticals, cosmetics, and packaging. Worldwide gelatin production exceeds 326,000 t per year, and more than three-quarters of this volume derives from mammalian sources—pig skin (≈46 %), bovine hide (≈29 %), and bovine/pig bones (≈23 %) (Gómez-Guillén et al. 2009). However, reliance on mammalian gelatin raises several concerns: the risk of transmissible diseases such as bovine spongiform encephalopathy, religious or cultural prohibitions, and consumer safety issues related to cross-contamination [8] (Gómez-Guillén et al. 2009). Consequently, the industry has turned increasingly toward fish-derived gelatin, which offers comparable functional properties—gel strength, rheology, emulsifying and foaming-forming abilities—while avoiding the drawbacks above [8][9] (Shiao et al. 2021; Gómez-Guillén et al. 2009).

The demand for gelatin has risen steadily, with an annual growth rate of 3–4 % over the past seven years [8] (Gómez-Guillén et al. 2009). Traditional fish gelatin is obtained from skins, bones, or fins of various species, including grass carp, yellowfin tuna, shark cartilage, Nile perch, channel catfish, cod head, Atlantic salmon, and flounder [10][11][12][13] (Kasankala et al. 2007; Cho et al. 2005; Arnesen & Gildberg 2006; Fernández-Díaz et al. 2003). Warm-water species tend to produce gelatin with higher proline and hydroxyproline content, resulting in superior tensile strength and water-vapour barrier performance, whereas cold-water species often yield gelatin with greater hydrophobicity [14] (Shakila et al. 2012).

Beyond functional attributes, fish gelatin exhibits bioactive properties such as antioxidant activity, antihypertensive effects, and promotion of wound healing, which broaden its applicability in nutraceuticals and medical devices [15] (Shankar et al. 2016; Ali Pal et al. 2020).

Despite these advantages, the utilisation of fish scales for gelatin production remains limited. Most scales are presently discarded or converted into low-value products (fertiliser, animal feed), representing a missed opportunity for value addition and waste reduction [4] (Arvanitoyannis et al. 2008). Conventional extraction methods employ strong acids, bases, or high-temperature hydrolysis, which can be environmentally damaging and energetically intensive [17][18] (Gilsenan & Ross-Murphy 1999; Holzer 1996). Recent research has demonstrated that proteolytic microorganisms isolated from fish-scale waste can hydrolyse collagen efficiently, yielding gelatin with yields up to 13 % (dry weight) and preserving key amide functional groups, as confirmed by FT-IR spectroscopy [6] (He et al. 2022). This microbe-mediated approach reduces the need for harsh chemicals, aligns with circular-economy principles, and offers a scalable pathway to transform scale waste into a high-value biopolymer.

The present study, therefore, aims to (i) isolate and identify gelatin-producing bacteria from Labeo rohita scales, (ii) optimise a microbial fermentation process for gelatin extraction, and (iii) characterise the physicochemical and functional properties of the resulting gelatin relative to commercial marine gelatin. By integrating waste valorisation with sustainable bioprocessing, this work seeks to provide an economically viable and environmentally responsible alternative to mammalian gelatin, supporting the broader transition toward greener materials in food, pharmaceutical, and packaging sectors.

MATERIALS AND METHODS

Sample Collection

Fish scale samples were collected from the Annan Koil Landing Centre fish request in Parangipettai, Cud-

dalore District, Tamil Nadu. The samples were transported to the laboratory in polyethene bags. They were completely washed using valve water followed by distilled water. After drying fully, the scales were stored in airtight plastic bags at 4 °C until further use.

Isolation of Bacteria (Cappuccino and Sherman, 2002)

One gram of fish scale samples was serially adulterated (10-fold dilutions). Also, 100 µL of the 10- 2, 10- 4, and 10- 6 dilutions were spread onto sterile Nutrient Agar plates and incubated at 37 °C for 24 hours. Distinct bacterial colonies were isolated and purified on Nutrient Agar plates. Five different isolates labelled as NAFS 10^{-2} A, NAFS 10^{-2} B, NAFS 10^{-2} C, NAFS 10^{-4} E, and NAFS 10^{-4} G were attained. These isolates were linked based on colony morphology, bitsy examination, and biochemical tests. Gelatin hydrolysis was tested for all five isolates. Birth of Bacterial societies. Bacterial societies grown in nutrient broth for 24 hours were centrifuged at 10,000 rpm for 15 minutes. The performing bullets were collected for further analysis, while the supernatant was discarded.

Alkali Treatment for Fish Scales (Das et al., 2017)

Dried fish scales were mixed with bacterial bullets in a 5% sodium chloride (NaCl) result at a rate of 110 and stirred for 30 twinkles. This step was repeated twice. Next, the scales were treated doubly with 0.4 M sodium hydroxide (NaOH), resulting in a 110 °C rate for 30 minutes in a shaking incubator. Following this, scales were treated three times with 10%M hydrogen peroxide (H_2O_2) at a 14 weight-to-volume rate for 30 twinkles each at 25 °C and 120 rpm to remove lipids. Demineralisation was performed by incubating the scales with 0.5 N EDTA and 3 mL of bacterial isolates at 14 °C for 16 hours in a shaking incubator. The scales were also irrigated with distilled water and soaked in 0.05 M acetic acid (14 rate) for 3 hours. Eventually, water was added at a 13 rate, and the mixture was dried in a hot air oven at 60 °C for 24 hours. The dried extract was ground into powder and placed at room temperature for further analysis.

Protein Estimation (Lowry Method, 1951)

Prepare the Lowry reagent by mixing 50 mL of Reagent A with 1 mL each of Reagent B and C until the mixture is clear. Prepare protein norms (e.g., BSA) by periodical dilution in distilled water (0.1 – 1.0 mg/ mL) to induce a standard wind. Pipette 0.1 – 1.0 mL of sample or standard into test tubes, add 5 mL of Lowry reagent, and mix well. Allow the admixture to stand at room temperature (20–25°C) for 10–15 minutes. Add 0.5 mL of Folin- Ciocalteu reagent to each tube and incubate for 30 – 60 minutes at room temperature. Measure absorbance at 640 nm using a spectrophotometer. Construct a standard curve by plotting absorbance versus protein concentration, and use it to determine the sample protein concentration.

RESULT

Isolation and Identification of Bacterial Cultures

Fish scale samples were serially diluted using ten-fold steps. Aliquots from selected dilutions were aseptically spread onto sterile agar plates inside a laminar airflow chamber to maintain sterility. These plates were incubated at 37°C to allow bacterial growth and facilitate colony enumeration. Phylogenetic analysis identified two dominant bacterial species isolated from the samples. The first isolate, label NAFS 10^{-2} A, was identified as *Aneurinibacillus aneurinilyticus*. This bacterium is a rod-shaped, endospore-forming, Gram-negative species. The 16S rRNA gene sequence was submitted to NCBI GenBank under accession number PV565637. The second isolate, NAFS 10^{-2} C, was identified as Enterobacter cloacae, a rod-shaped, facultative anaerobic, Gram-negative bacterium. Its 16S rRNA sequence was submitted to NCBI GenBank with accession number PV565627. The phylogenetic positions of these isolates were confirmed through sequence comparison and analysis, supporting their taxonomic classification.

FIGURE 1: Shows a purified bacterial culture

Table 1. Polymerase Chain Reaction (PCR)-168 Gene Application

	PRIMERS
27F	AGAGTTTGATCMTGGCTCAG
1492R	TACGGYTACCTTGTTACGACTT

PCR Amplification and Gel Electrophoresis Analysis

PCR amplification of bacterial DNA extracted from fish scale samples FS A and FS C was confirmed by agarose gel electrophoresis. The gel image included the following lanes: Lane 1 and Lane 4 contained the 100 bp DNA ladder, which consists of DNA fragments ranging from 100 bp to 1.5 kb, serving as molecular size markers. Lane 2 showed the PCR product from samples FS A and FS C, and Lane 3 contained the positive control to verify the PCR reaction's reliability.

The DNA bands in Lane 2 appeared at the expected size, consistent with the target gene amplification, demonstrating the successful amplification of bacterial DNA from the fish scale samples. Comparison with the ladder bands confirmed that the amplified fragments fell within the anticipated base pair range, validating the presence of the specific bacterial DNA sequences under study.

These results confirm the specificity and efficiency of the PCR protocol used for detecting bacterial species in fish scales.

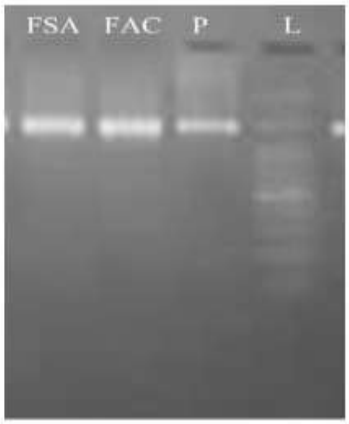


FIGURE 2: Shows Lane 1: FS A; Lane 2: FS C; Lane 3: Positive control; Lane 4: Ladder

DNA L100bp DNA Ladder

100 bp Ladder contains 11 DNA fragments of size 100 bp, 200 bp, 300 bp, 400 bp, 500 bp (Spiked), 600 bp,700 bp, 800 bp, 900 bp, 1 kb, and 1.5 kb.

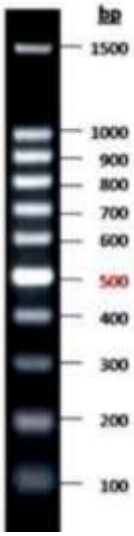


FIGURE 3: Shows a 100bp DNA ladder

Gelatin was successfully extracted from the fish scales using a series of pretreatment steps involving alkali and acid soaking by hot water extraction. The extracted gelatin appeared as a pale yellow, odourless sheet after drying. The overall yield of gelatin obtained from the fish scales is shown in Table 2.

FIGURE 4: Shows a Final Gelatin Extracted powder

Yield of Gelatin with Microbe-Mediated Extraction

The yield of gelatin was calculated based on the dry weight of the fish scale by using the following equation.

Yield of gelatin (%) = weight of dried gelatin (g) / Dry weight of fish scales (g) × 100

Table 2: The yield of extracted gelatin was calculated by the above-given equation

NAME OF THE SAMPLE	NET WEIGHT OF DRIED SAMPLE	YIELD OF GELATIN EXTRACTED (%)
NA FS 10^{-2} (A)	5g	13.2%
NA FS 10^{-2} (B)	5g	12.96%
NA FS 10^{-2} (C)	5g	11.92%
NA FS 10^{-4} (E)	5g	12.42%
NA FS 10^{-4} (G)	5g	9.72%

Protein Percentage and p^H Range of Gelatin

The protein percentage of extracted microbe-mediated gelatin was determined using a UV-Spectrophotometer, and P^H was measured using a P^H meter.

Table 3: P^H and Protein% of extracted gelatin were compared with commercial marine gelatin.

SAMPLE	OD VALUE	PROTEIN%	P^H	COMMERCIAL GELATIN PROTEIN%	COMMERCIAL GELATIN (P^H)
A	0.0902	8.1%	5.71	8.5%	6.43
B	0.1068	9.6%	5.45		
C	0.0774	7%	5.57		
E	0.1334	12.06%	5.49		
G	0.1042	9.42%	5.70		

Pie Chart For Yield And Protein Percentage of Extracted Gelatin

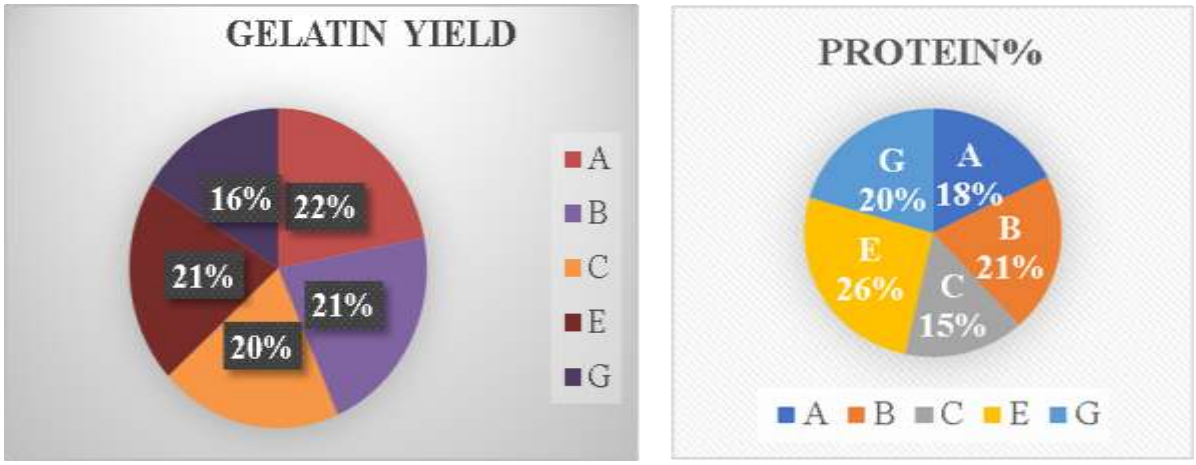
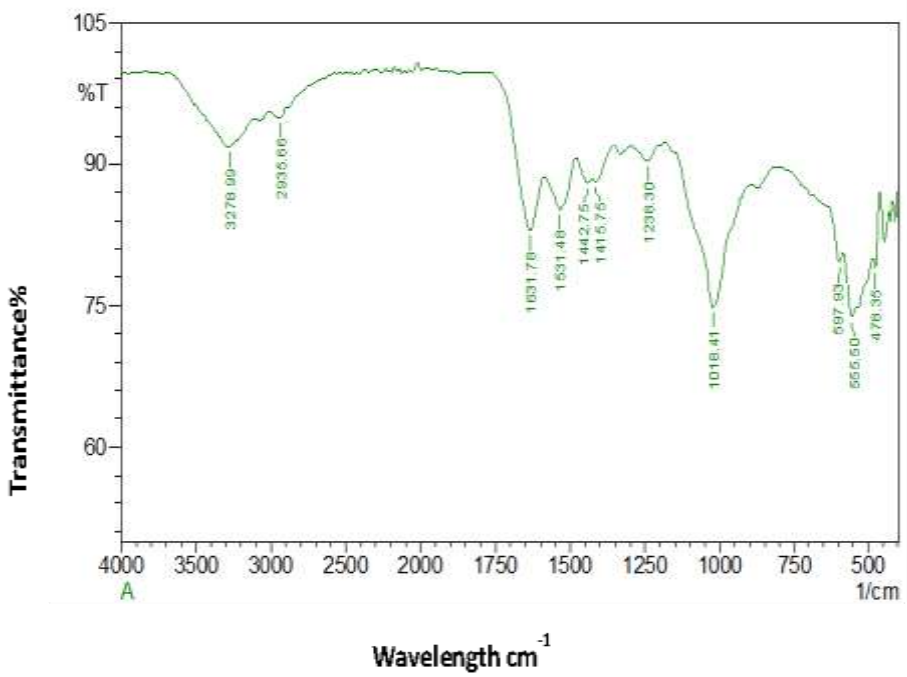


FIGURE 5: Shows a Pie Chart For Yield And Protein Percentage of Extracted Gelatin

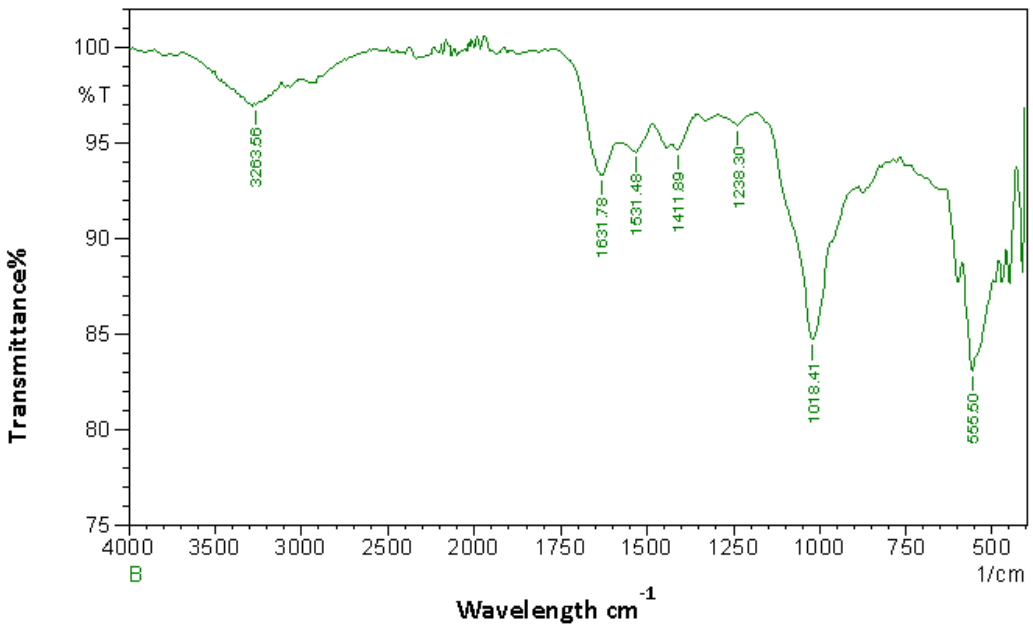
Fourier Transform Infrared (FTIR) Spectroscopy Analysis

FTIR analysis using the ATR method revealed characteristic peaks of gelatin. A broad peak observed between 3300 and 3500 cm^{-1} corresponds to hydrogen-bonded water and the amide A group. The peak at 1631 cm^{-1} is attributed to amide I, while the peak at 1531 cm^{-1} indicates amide II. The band at 1238 cm^{-1} represents the amide III band. Additionally, peaks in the range of 1415 to 1442 cm^{-1} were assigned to

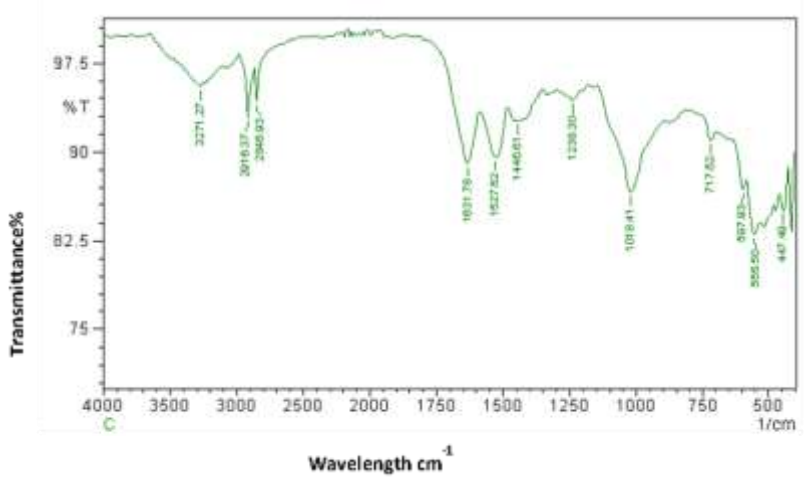
symmetric and asymmetric bending vibrations of the methyl groups. These spectral features confirm the presence of gelatin and its typical molecular structure.



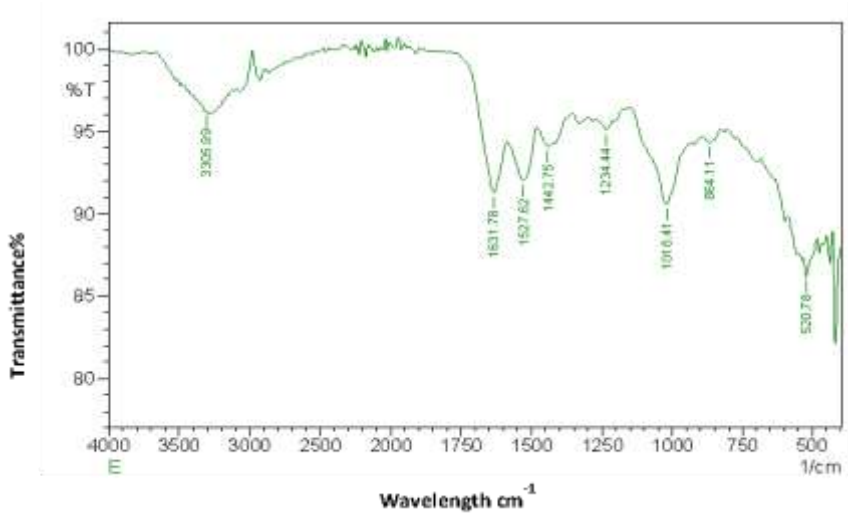
Graph 1: Shows a characterisation of NAFS10^{-2} (A) extracted gelatin via FTIR



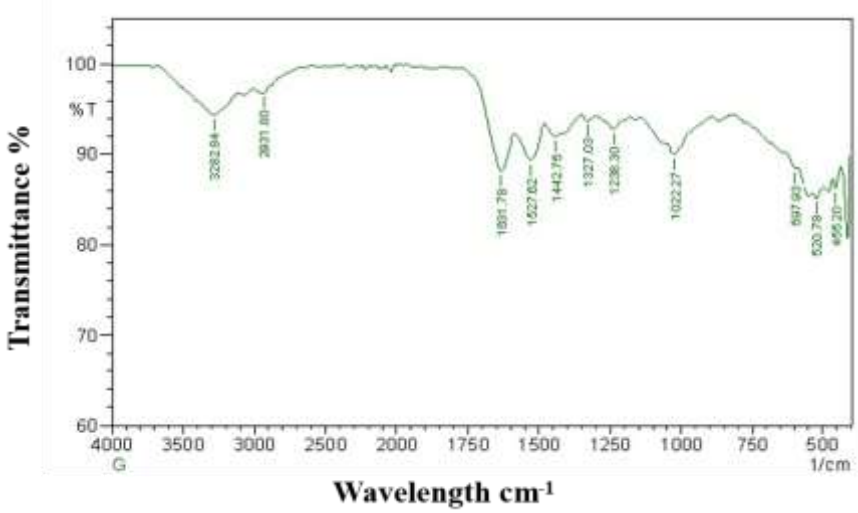
Graph 2: Shows a characterization of NAFS10^{-2}(B) extracted Gelatin via FTIR analysis



Graph 3: Shows a characterisation of NAFS10^{-2} (C) extracted gelatin via FTIR analysis



Graph 4: Shows a characterisation of NAFS10^{-4}(E) extracted gelatin via FTIR analysis.



Graph 5: Characterisation of NAFS10^{-4} (G) extracted gelatin via FTIR analysis.

DISCUSSION

This study successfully demonstrated the potential of converting fish scale waste into valuable gelatin using bacterially assisted extraction. Five distinct bacterial strains [NAFS 10^{-2} (A), NAFS 10^{-4} (B), NAFS 10^{-2} (C), NAFS 10^{-4} (E), and NAFS 10^{-4} (G)] exhibiting proteolytic activity via gelatin hydrolysis were isolated, highlighting their crucial role in gelatin degradation. This approach aligns with previous findings from Lahore, Pakistan, where researchers emphasised the importance of converting waste materials into useful products to mitigate environmental pollution (Rabia Anwar Rao et al. 2022). Similar bacterial isolates, which demonstrated gelatin hydrolysis, were recovered from the Labeo rohita fish species in that study.

Morphological, biochemical, and microscopic analyses suggested that the isolated bacteria are closely related to *Aneurinibacillus aneurinilyticus* and *Enterobacter cloacae*. Previous studies from Lahore also reported involvement of Pseudomonas, Staphylococcus, and Bacillus species in gelatin hydrolysis (Rabia Anwar Rao et al. 2022). The pH values of gelatin extracted using 0.05 M acetic acid ranged between 5.45 and 5.80, which is somewhat lower than the pH of commercial gelatin (6.43). This value is comparable to those reported for fish scale gelatin in India, which showed a pH of around 4.35 (Merina Paul Das et al., 2017).

Including bacterial isolates during the demineralisation step with 0.5 N EDTA effectively removed inorganic materials to yield a purer protein product. Protein content measured by the Lowry assay ranged from 7% to 12.6% per 100 mg of extracted gelatin, comparable to protein levels seen in commercial marine gelatin sources (~8.5%) (Benjamaporn Wonganu 2020). The optimised extraction method enhanced eco-friendliness by reducing reliance on harsh chemicals, notably replacing conventional lipid solvents with 10% hydrogen peroxide. While bacterial isolates likely contribute hydrolytic enzymes that facilitate scale breakdown, further genomic studies are necessary to identify the specific enzymatic pathways involved.

FTIR analysis confirmed the presence of characteristic gelatin functional groups and secondary structures. Broad peaks between 3282 and 3500 cm^{-1} suggested hydrogen bonding with water and Amide A groups. Peaks at 1631 cm^{-1} (Amide I), 1527 cm^{-1} (Amide II), and 1238 cm^{-1} (Amide III) corresponded to typical protein amide bonds. Additionally, the range of peaks from 1442 to 1327 cm^{-1} was assigned to methyl group bending vibrations. These spectral features are consistent with gelatin gel properties described elsewhere, such as in Switzerland (Nisakorn Nuamsrinuan et al. 2015).

The gelatin yield from this study ranged from 9.72% to 13.2% from 5 g of fish scale samples, which is in line with previous reports describing significant yields of fish scale gelatin (Rabia Anwar Rao et al. 2022). This supports the feasibility of bacterially assisted gelatin extraction from fish by-products, presenting a sustainable method for waste valorisation in aquaculture industries.

SUMMARY AND CONCLUSION

This study explored the microbial-assisted extraction of gelatin from fish scales, focusing on optimising yield, analysing physicochemical properties, and comparing results with commercial gelatin. Two bacterial species, *Aneurinibacillus aneurinilyticus* and *Enterobacter cloacae*, were isolated and found to contribute significantly to collagen degradation during the extraction process.

Gelatin yields varied between 9.72% and 13.2%, with the highest yield from NA FS 10^{-2} (A). Protein content ranged from 7% to 12.06%, and the extracted gelatin showed slightly acidic pH values (5.45–5.71), which were lower than those of commercial marine gelatin. FTIR analysis confirmed key functional groups characteristic of gelatin, supporting the preserved structural integrity of the product.

These findings demonstrate the feasibility of using bacterial isolates to produce gelatin from fish scale waste with promising physicochemical properties, offering a sustainable alternative to traditional gelatin extraction methods. Further work is needed to optimise bacterial strains, scale up the process, and enhance gelatin functionality for industrial applications.

ACKNOWLEDGEMENT

We sincerely thank the Centre of Advanced Study in Marine Biology, Annamalai University, Parangipettai, for their generous support, research facilities, and valuable guidance that greatly aided in the successful completion of this work.

REFERENCES

1. Fagbenro, O.A., et al. (2005). Alternative Protein Sources for Farmed Tilapia. Aquaculture Nutrition, 11(2), 93-104.
2. Usman, M., et al. (2022). Fish protein hydrolysates as health-promoting ingredients: recent update. Nutrition Reviews, 80(5), 1013-1026.
3. Kelleher, K. (2005). Discards in the world's marine fisheries: An update. FAO Fisheries Technical Paper, No. 470. Food and Agriculture Organisation of the United Nations.
4. Arvanitoyannis, I.S., Kassaveti, A. (2008). Fish industry waste: treatments, environmental impacts, current and potential uses. International Journal of Food Science & Technology, 43(4), 726-745.
5. Vannuccini, S. (2004). Overview of fish production, utilisation, trade, and consumption. In: World Fisheries and Aquaculture Atlas. FAO.
6. He, P., et al. (2022). Extraction and industrial valorisation of collagen, gelatin, and bioactive peptides from freshwater fish scales: A review. Trends in Food Science & Technology, 128, 73-83.
7. Shyni, K.S., et al. (2013). Fish scale collagen: a biomaterial for future biomedical applications. Current Organic Chemistry, 17(4), 647-655.
8. Gómez-Guillén, M.C., et al. (2009). Functional and bioactive properties of collagen and gelatin from alternative sources: A review. Food Hydrocolloids, 23(8), 1768-1784.
9. Shiao, M.C., et al. (2021). Advances in marine gelatin and its application in food, pharmaceutical, and biomedical industries. Marine Drugs, 19(6), 290.
10. Kasankala, L.S., et al. (2007). Fish gelatin extraction and characterisation from different species. Food Chemistry, 101(2), 603-609.
11. Cho, S.Y., et al. (2005). Preparation and characterisation of gelatin from skipjack tuna (Katsuwonus pelamis) scales. Food Hydrocolloids, 19(4), 645-652.
12. Arnesen, J.A., Gildberg, A. (2006). Raw material and process modifications of gelatin from cod skin. Food Hydrocolloids, 20(5), 630-636.
13. Fernández-Díaz, M., et al. (2003). Characterisation of gelatin from fish by-products. Food Chemistry, 79(2), 247-255.
14. Shakila, R.J., et al. (2012). Properties and potential applications of fish gelatin from cold-water and warm-water fish species: a review. Food Hydrocolloids, 26(1), 1-17.
15. Shankar, S., et al. (2016). Bioactive properties of fish gelatin hydrolysates: A review. Journal of Food Science and Technology, 53(2), 354-359.
16. Ali Pal, A., et al. (2020). Fish gelatin and its wound healing properties: a review. Biotechnology Reports, 25, e00428.

17. Holzer, A. (1996). Gelatin: The backbone in pharmaceutical capsules. Drug Development and Industrial Pharmacy, 22(9), 797-820.
18. Gilsenan, P.M., Ross-Murphy, S.B. (1999). Rheological characterisation of gelatin gels: a review. Food Hydrocolloids, 13(4), 455-467.
19. Joy, J.M., Pandey, A., & Kumar, S. (2024). A Review on Fish Skin-Derived Gelatin: Preparation, Bioactive Properties, and Industrial Applications. Molecules, 29(18), 5377.
20. Patwary, M.A., et al. (2025). Extraction and Industrial Valorisation of Collagen, Gelatin, and Hydroxyapatite from Freshwater Fish Scales: A Comprehensive Review. Trends in Food Science & Technology, 128, 73-83.
21. Rao, R.A., et al. (2022). Utilisation of Fish Scales for Microbe-Mediated Gelatin Extraction: A New Biotechnology Avenue. Turkish Journal of Fisheries and Aquatic Sciences, 22(12), 1070-1082.
22. Zhang, Y., et al. (2019). Fish Scale Valorisation by Hydrothermal Pretreatment and Enzymatic Hydrolysis for Gelatin Production. Marine Drugs, 17(8), 432.
23. Wonganu, B. (2020). Valorisation of Fish Scale Waste for Gelatin Production: Characterisation and Applications. Food Bioscience, 34, 100545.
24. Ranasinghe, R., et al. (2023). Functional and Bioactive Properties of Gelatin Extracted from Aquatic Sources: A Review. Food Hydrocolloids, 138, 108382.
25. Lu, W.C., et al. (2023). Characterisation and Biological Properties of Marine By-Products: Marine Collagen Extraction and Application. Marine Drugs, 21(2), 84.
26. Shakila, R.J., et al. (2012). Properties and Potential Applications of Fish Gelatin from Different Aquatic Resources. Food Hydrocolloids, 29(1), 109-117.
27. Sreeja, S. J., Satya, J., Tamilarutselvi, K., Rajajeyasekar, R., Tamilselvi, A., Nandhakumari, P., & Sarojini, K. (2024). Isolation of gelatin from fish scale and evaluation of chemical composition and bioactive potential. Biomass Conversion and Biorefinery, 14(18), 22161-22170.