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Formation, characterization of gelatine from the scales of *Labeo rohita* and its comparison with bovine bone gelatine

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Abstract

The study isolated gelatin from *Labeo rohita* scales and characterized it using Fourier transform infrared spectroscopy (FTIR), X-Ray diffraction (XRD), and scanning electron microscopy (SEM). The resulting gelatine and commercial bovine bone gelatin were found to have crystalline structures, with both being porous and spherical. *L. rohita* scales yielded 5.45 % of gelatin. The moisture content varied between 11.12 % and 9.02 % for the gelatin, while the ash content varied between 2.17 % and 2.36 %. The protein content was 82.78 % in the fish scales gelatin, while the commercial bovine bone gelatin had 94.27 %. The fat content of gelatin isolated from scales of *L. rohita* was 1.21 %, whereas fat in commercial bovine bone gelatin was 1.19 %. The fiber contents of gelatin isolated from fish scales was 0.44 % while fiber content of commercial bovine bone gelatin was 0.65 %. The study confirms the high-quality gelatin-bearing characteristics of fish scales, suggesting that it can be produced for various purposes and potentially increase the economic value of fish. The isolated gelatin from fish by-products could also be a valuable resource.

Introduction

"Gelatin" originates from the Latin word "gelatus", which means frozen or firm (Khan 2020). It is a polypeptide polymer shaped into a fibrous structural protein that can be obtained from the degeneration and partial hydrolysis of collagen from animal tissues (Kamer *et al.* 2019; Cheng *et al.* 2019). Commercially, it is found in the form of tablets, granules, or powders, and sometimes it can be dissolved in water before use (Yang *et al.* 2016). As gelatine is the most abundant and non-toxic (biodegradable, biocompatible, and soft) source of protein (Portier *et al.* 2017; Huang *et al.* 2019), it is widely used in different food items (Huang *et al.* 2019; Cheng *et al.* 2019; Ishaq *et al.* 2020), pharmaceuticals (Cebi *et al.* 2016; Lin *et al.* 2017), cosmetics (Eaqub *et al.* 2018) and photography industries (Lin *et al.* 2017; Tümerkan *et al.* 2019). Currently, the market is dominated by mammalian

(beef and pork) gelatin (~98.5 %) (Huang *et al.* 2019) however, consumption from these animal sources contradicts ethnocultural and religious norms and is associated with the risk of contracting prion diseases (Karim and Bhat 2008, 2009; Cheng *et al.* 2018; Huang *et al.* 2019; Ishaq *et al.* 2020). The religious sentiments and the anxiety of consumers against mammalian gelatin have compelled producers to consider alternate sources (Karim and Bhat 2009; Duan *et al.* 2018). As a protein source, fishes are the most uncontroversial resources, hence goes true to gelatin as well (Liu *et al.* 2015; Lin *et al.* 2017; Cheng *et al.* 2019). In addition, fish gelatin shares novel potential applications such as antihypertensive, antioxidant, antimicrobial, tissue regeneration and wound healing promotion, bone formation enhancement, anticancer applications, anti-adhesion applications, applications in gene therapy, and applications in antimicrobial packaging (Lv *et al.* 2019). Therefore, as an alternative to the mammalian ones, gelatin has been extracted successfully from fish skin (Giménez *et al.* 2005; Taheri *et al.* 2009; Wu *et al.* 2013; Fan *et al.* 2017; Tkaczewska *et al.* 2018; Wang *et al.* 2020), bone (Muyonga *et al.* 2004c; Taheri *et al.* 2009; Qiu *et al.* 2019), scales (Sha *et al.* 2014; Tu *et al.* 2015; Chen *et al.* 2018; Sreeja *et al.* 2023), swim bladders (Kaewdang and Benjakul 2015), cartilages (Cho *et al.* 2004; Zhang *et al.* 2022) and fish heads (Elavarasan *et al.* 2017). Still, extraction of gelatin from different fishes inhabiting varied geographical and environmental conditions is useful, as the functional properties of gelatin are greatly influenced by the amino acid composition, the molecular weight distribution (Kołodziejska *et al.* 2008; Muyonga *et al.* 2004a; Muyonga *et al.* 2004b) and the ratio of α/β chains present in the gelatin (Karim and Bhat 2009).

Consumption of fish leads to the generation of large volumes of biomass, which includes viscera, skin, bones, scales, fins, and other muscles (Karayannakidis and Zotos 2016). Fish waste is, therefore, a good reservoir of collagen protein and can act as an alternative source for gelatin production. A total of 7.2 – 12 million tons of fish waste are discarded every year (Qin *et al.* 2022). Currently, there is no evident commercial utilization of fish wastes especially fish scales (Nawshad *et al.* 2016; Marrakchi *et al.* 2017). The landfill disposal of fish scale waste may cause serious environmental pollution problems and lead to a waste of resources (Hoyer *et al.* 2012). Altogether, gelatin extraction from scales would therefore be an effective way of utilising fish waste. Alongside, *Labeo rohita* (Rohu) is one of the major Indian carps that occupies an important place in aquaculture in Southeast Asia, such as Bangladesh, India, Myanmar, Vietnam, Pakistan, Laos, and Nepal (Shahjahan *et al.* 2021). *Labeo rohita* (Rohu), an economically important and highly consumable fish due to its delicious taste in the fish markets of India and Pakistan (Bhatkar 2011). Second, to the best of our knowledge, these nations have not reported any comparative studies on the characteristics of gelatin derived from *L. rohita* scales. In accordance with this, the current investigation aimed to isolate gelatin from *L. rohita* scales and compare their physicochemical characteristics in relation to easily accessible bovine bone gelatin.

Experimental

Collection of scales and extraction of gelatin

For the collection of scales, *L. rohita* fish were procured fresh from the local fish farm in district Kohat, Khyber Pakhtunkhwa, Pakistan. The fish scales were removed by a scalpel, packed in zip-lock plastic bags, frozen, and immediately cleaned with distilled water, dried, and stored at ambient temperature. Further, 1,100 g dried scales were treated with a 0.1 M NaOH solution (dried fish scales: solution = 1 : 15, w/v) and stirred for 6 h at room temperature to remove unnecessary proteins. After every three hours, the solution was changed, as mentioned earlier (Chuaychan *et al.* 2016). Later, the scales were washed thoroughly with distilled water until they became neutral or slightly alkaline. Thereafter, the scales were demineralized with a 0.4 M HCl solution for 90 minutes (Zhang *et al.* 2009) and then rinsed with distilled water until become mildly acidic. Isolation of gelatin from the scales was performed by following the method (Yang *et al.* 2009), with minor modifications. Distilled water (dried fish scales: water = 1 : 9, w/v) was added to the scales and heated at 121 °C for 2 h using an autoclave. The resulting solution was filtered through cheesecloth.

Proximate and yield analysis of gelatin

The proximate composition of gelatin, such as moisture, ash, protein, and fat contents, was analysed using the standard methods of the Association of Official Analytical Chemists (AOAC, 2000). Further, for yield analysis of gelatin based on the weight of dry scales, the formula (Eq. 1) of (Thanasak and Benjakul 2015) was used which is as follows:

$$\% \text{ yield (dry weight basis)} = \frac{\text{Weight of dried gelatin(g)}}{\text{Dry weight of fish scale(g)}} \times 100 \quad (1)$$

pH or pH analysis of gelatin

The gelatin pH was estimated using the pH meter following the method mentioned by (Das 2017). The pH of the gelatin solution was determined by preparing 1 % (w/v) gelatin solution in distilled water and keeping it at 25 °C in a water bath, and pH was measured.

Fourier Transform Infrared (FTIR) Spectroscopy Analysis

The FTIR spectrophotometer (Bruker-Tenson 37) was used to analyze the chemical composition of gelatin in order to identify the major functional groups in charge of gelatin reduction and stabilization as well as the chemical and structural makeup of gelatin powder in the 4,000 – 500 cm^{-1} range.

X-Ray diffraction analysis of gelatin

The gelatin powder was further characterized using the X-ray diffraction technique. It was carried out in an X-ray diffractometer (X'Pert Pro A Analytical) operated at 45 kV voltage and 40 mA current. The pattern was recorded by Cu Kα radiation in a θ – 2θ configuration.

Scanning Electron Microscopy (SEM) Analysis

The morphological features of gelatin were studied by scanning electron microscope (FEI Quanta 200 SEM). The surface of dried gelatin was coated with gold in a vacuum using sputter coater and was photographed.

Results and Discussion

Fourier transform infrared (FTIR) spectroscopy analysis

The FTIR spectra are used to reveal the secondary structure of proteins and to observe the changes in protein structure based on hydrogen bonding (Wang *et al.* 2020). So, this study found all four peaks for Amide I, Amide II, Amide III, and Amide A in fish scale gelatine as normally found in bovine gelatine (Fig. 1). The peaks for amides I, II, and III appeared at 1,645 cm^{-1}, 1,537 cm^{-1}, and 1,238 cm^{-1} for isolated fish scale gelatins and peaks appeared at 1,632 $cm^{-1,}$ 1,531 $cm^{-1,}$ and 1,231 cm^{-1} for commercial bovine bone gelatin. The literature also supports major absorption bands at 1,600-1,700 cm^{-1} for Amide I, representing C–O stretching, 1,500 – 1,550 cm^{-1} for Amide II representing N–H deformation, and 1,200 – 1,300 cm^{-1} for Amide III, representing C–N stretching and N–H deformation (Zhao *et al.* 2021) Amide A represents the free N–H stretching vibration in the range of 3,400 to 3,440 cm^{+-1} (Li *et al.* 2013; Khan 2020). The wavenumber shifts to a lower frequency when the N–H group of the peptide is involved in hydrogen bonding (Zhao *et al.* 2018). Similarly, amide A peak of isolated fish scale gelatin appeared at 3,296 cm^{-1} whereas amide A peak of commercial bovine bone gelatin appeared at 3,282 cm^{-1}, hence both are comparable. (Fig. 1 shows that the amide A wavenumbers of *L. rohita* (3,296 cm^{-1}) and commercial bovine bone (3282 cm^{-1}) indicate that some N–H groups in fish scales gelatin and commercial bovine bone contributed to the formation of hydrogen bonds, and the degree of hydrogen bonding in commercial bovine bone gelatin was greater than that of fish scales gelatin. On the other hand, the fish scale gelatin showed a strong absorption peak at 1,021 cm^{-1} and

commercial bovine bone gelatin at 1,076 cm^{-1} assigned to asymmetric stretching of the phosphate group (Sankar *et al.* 2008; Panda *et al.* 2014). The FTIR data of fish scales gelatin in our study were compared with commercial bovine bone gelatin, and the data reveals that *L. rohita* fish scales gelatin showed more desired peaks and did not vary significantly from commercial bovine bone gelatin, which showed that gelatin from *L. rohita* scales could be used as a potential replacement for commercial bovine bone gelatin.

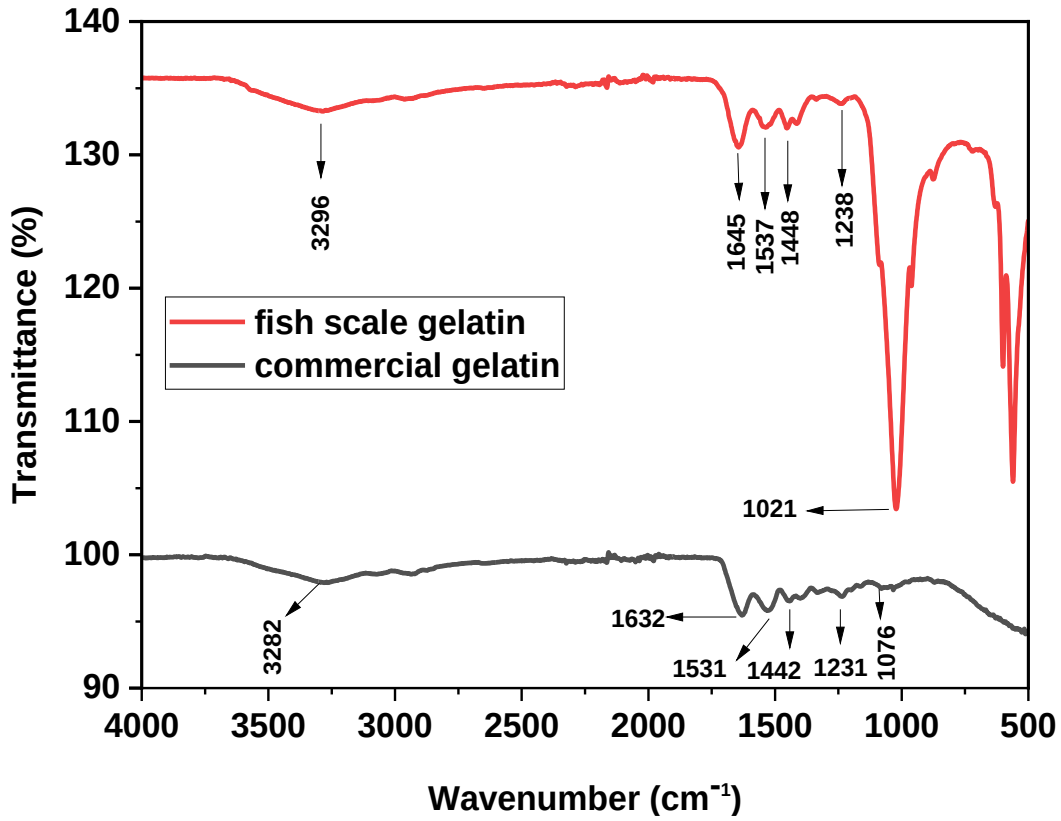


Fig. 1. FTIR spectra of fish scale gelatin and commercial gelatin/Bovine bone gelatin. The red line shows the peak of fish scale gelatin and the grey line shows the peak of commercial bovine bone gelatin.

X-Ray diffraction analysis of gelatin

XRD analysis enables us to study the structure of a material. The degree of crystallization in the polymers was detected by X-ray diffraction. We analysed the isolated fish scale gelatin that shows crystallinity with one diffraction peak, namely, the No. 1 peak at 2θ = 10° and the No. 2 peak at 2θ = 31 (Itoh *et al.* 1994; Peña *et al.* 2010) while commercial bovine bone gelatin is partially crystalline in nature and shows a broad peak at 2θ = 20 (Rivero *et al.* 2010). The peak at 7.0 – 8.1° in gelatin is typically assigned to the triple-helical crystalline structure (Peña *et al.* 2010). The absence of this peak in the XRD pattern of fish scale gelatin and commercial bovine bone gelatin could be due to the reduction of hydrogen bonding between polymer molecules in the presence of water and heat during sample preparation.

Differences in crystal structure reflect the position of diffraction peaks. As it can be seen, the position and intensity of those diffraction peaks of fish gelatin are changing. This shift could be the result of the variation in the gelatin origin and the moisture content of the gelatin samples (Díaz-Calderón *et al.* 2017). It should also be noted that as the time duration of the extraction increased, the intensity of the peak also increased. Gelatin is amorphous as well as crystalline in structure, so variation in the above parameter or the intermolecular interaction of gelatin may induce new crystalline peaks in gelatin, which result in the modification of the crystallinity

of the gelatin. The significance of crystalline gelatin is that it is more thermally stable and can maintain a very high temperature as compared to semi-crystalline or amorphous gelatin because crystalline gelatin has a high melting and boiling point.

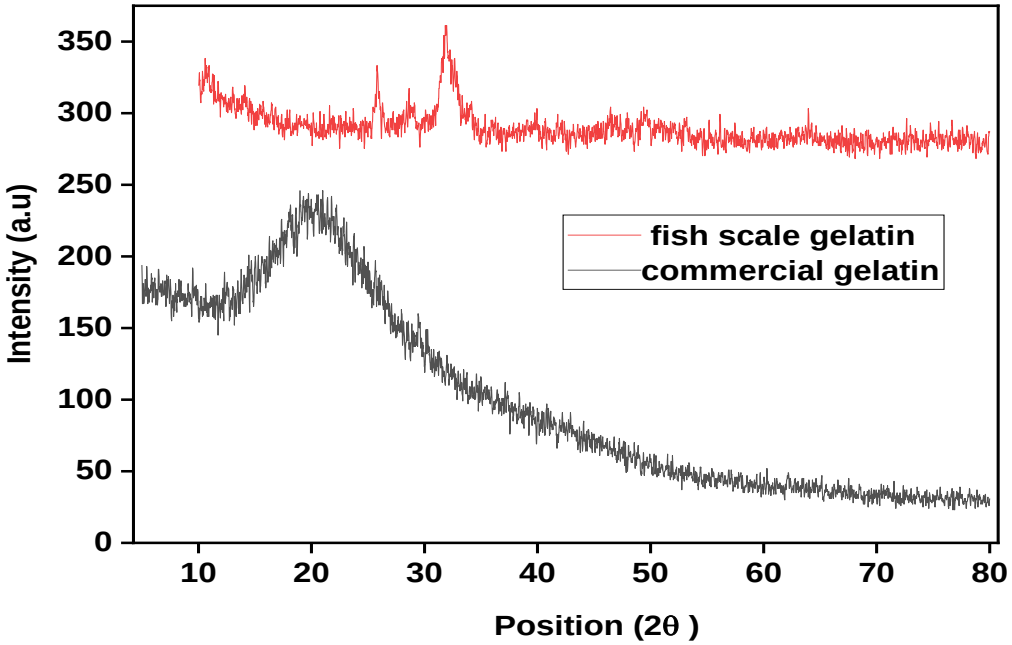
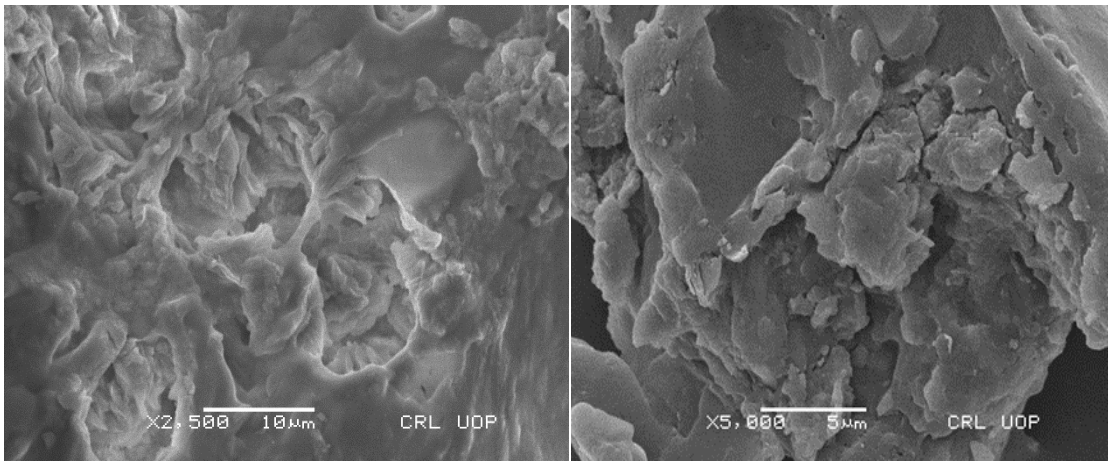


Fig. 2. XRD spectra of fish scale gelatin and commercial gelatin/bovine bone gelatin.

Scanning electron microscopy (SEM) analysis

The microstructures of the obtained isolated fish scale gelatin powder were examined by scanning electron microscopy (SEM) and compared with commercial bovine bone gelatin as shown in Fig. 3. The morphology of fish gelatin showed a sheet-like fibrous, porous nature, and voids with rough surfaces were observed, while in commercial gelatin the cells are tightly packed and aggregated with rough surfaces. As the mineral present in the scale was partially dissolved by acid, the porous nature of the sheet is seen in the isolated fish scale gelatin (Sankar *et al.* 2008). (Ratnasari *et al.* 2013) investigated the microstructure of the gelatin from the skin of different freshwater fishes via SEM micrographs and reported the non-uniform network in commercial gelatin, the denser strand with small pores in catfish gelatin, the slight strand in Nile tilapia gelatin, and the snakehead network and rough structure observed in Asian redtail catfish gelatin. Correspondingly, (Sreeja *et al.* 2023) reported rough surface morphology, a uniform network, and small voids. The extraction of gelatin at higher temperatures is reported to have larger voids in the matrix, which indicates the consistent distribution of water in the fine and ordered matrix. The thick network of irregular fibrillar structures represents the presence of ionic, hydrogen bonds, and hydrophobic interactions in the protein molecular matrices (Nomura *et al.* 2000). The high protein concentration of the gelatin showed a rough surface in the SEM micrograph, which evidenced intermolecular interactions and cross-linking development subsequent to the gelatin with high mechanical strength and brittleness (Fan *et al.* 2020). The interesting porous and rough nature of gelatin we report from scales of fish can be better applicable for various purposes in drug deliveries and encapsulation of active ingredients. Moreover, the porosity of our extracted gelatin may also provide channels for fluid penetration, allowing for efficient diffusion or permeability in certain applications. The hollow and porous gelatin may be used for desired purposes, depending on the specific requirements of the product or process.



a) Fish scale gelatin **b)** Commercial gelatin

Fig. 3. SEM micrographs of fish scales gelatin (**a**) and commercial gelatin/bovine bone gelatin (**b**).

Proximate composition of gelatin

Yield of gelatin

The proximate composition of isolated fish scale gelatin and commercial bovine bone gelatin is shown in Table 1. The yield of isolated light yellow-coloured gelatin was 17 % based on the dry weight basis of fish scales. The gelatin yield of the present study was higher than previously reported by (Jamilah and Harvinder 2002), who isolated 7.81% of gelatin from the skin of red tilapia and 5.39 % from black tilapia. Similarly, Cheow *et al.* (2007) extracted gelatin yield from the skin of sin croaker and shortfin scad as 14.3 % and 7.25 % respectively. (Jongjareonrak *et al.* 2010) reported a gelatin yield of 20.1 g/100 g based on the wet weight of the skins of giant catfish. Das (2017) reported a gelatin yield of 24 % from the scales of *L. rohita*, whereas (Sreeja *et al.* 2023) reported a gelatin yield of 25 % from the scales of *L. rohita*. The difference in yield of gelatin (mainly protein) could be due to the extraction process, temperature, and pH and could vary from species to species (Silva *et al.* 2014). The yield of gelatine could also be different because of the environment and species habitats that sequester the structures and physical properties of gelatines (Nurilmala *et al.* 2022). This relatively lower yield of gelatin in our study could also be due to the loss of extracted collagen through leaching during the series of washing steps or to incomplete hydrolysis of the collagen (Jamilah and Harvinder 2002). Other factors that influence the yield and quality of gelatin are the total protein concentration of the fish, the selection of the sample and species, and the age of the fish (Karim and Bhat 2009; Jongjareonrak *et al.* 2010; Koli *et al.* 2011). Further work is needed to improve this yield and quality of gelatin from the scales of fish, which may hint directly towards commercialization (Table 1).

Moisture analysis

The moisture content of fish scales gelatin was 11.12 %, while the moisture content of commercial bovine bone gelatin was 9.02 %, which is an acceptable range (15 %) for edible gelatin (GME 2005). Moisture content in the gelatin from scales of sea bream showed a moisture content of 9.69 % (Akagündüz *et al.* 2014). Similarly, Cao *et al.* (2017) explored the moisture content of gelatine from the skin of European perch and Volga pikeperch at 10.8 % and 8.2 %, respectively. (Shyni *et al.* 2014) reported the moisture level in shark, rohu, and tuna skin derived gelatines, and the results were found to be 8.7, 9.3, and 10.9 %, respectively. The moisture content we report in the gelatine from scales of *L. rohita*, along with previously reported gelatin from various sources, is quite below the range that affects the gelling ability, shelf life, and texture of the gelatin (Table 1).

Ash analysis

The ash content of gelatin fish scales was 2.17 % while the ash content of commercial bovine bone gelatin was 2.36 % in this study. Our result agreed with Sreeja *et al.* (2023) who also reported an ash

content of 2.1 % in gelatin from scales of *L. rohita*. Similarly, the ash content of gelatin from sea bass was 1.43 % and that of grey mullet was 1.55 % (Cao *et al.* 2017). In addition, (Qiu *et al.* 2019) reported the ash content as 1.05 % in the gelatin from scale from skipjack tuna. The ash content of isolated fish scales gelatin was lower than that of commercial bovine gelatin, which might be due to the difference between their raw materials. Ash content could be affected by the washing process, and the lower ash content indicates that most minerals have been washed off (Nurilmala *et al.* 2022). The ash content of bovine bone gelatin is higher than the gelatin isolated from fish scales. However, these values were considered low for fish gelatin, as the maximum advised amount of ash content in gelatin is 2.6 % (Alfaro *et al.* 2013) (Table 1).

Protein analysis

The isolated fish scale gelatin shows protein a content of 82.78 %, while commercial bovine bone gelatin showed a protein content of 94.27 %, as protein is the main constituent of gelatin. Meanwhile, the protein level in the extracted gelatin is similar (82.78 %) compared to the protein contained in lizard fish scales gelatin (86.90 %) reported by Wangtueai and Noomhorm (2009). Likewise, Jamilah *et al.* (2011) explored the protein content of 93.25 % in the gelatin from the skins of red tilapia, 77.88 % in walking catfish, and 80.02 % in striped catfish. The protein content of gelatin extracted from the scales of *L. rohita* was 90 % (Das 2017). The proximate composition of the gelatin in the fishery products is species-specific, varies with the sex and age of the fish, is seasonally varied, and varies with feeding habits (Silva *et al.* 2014). Taking all the reports of protein content in the gelatin together, the variations are probably due to the differences in the method of preparation of gelatin (Gómez-Guillén *et al.* 2011) (Table 1).

Fat analysis

The fat content of fish scale gelatin was 1.21 %, while the fat content of commercial bovine bone gelatin was 1.19 %. In the present study, the fat content in gelatin was higher than scales of sea bream (0.1 %; (Fahmi *et al.* 2004)) and lizardfish scales (0.067 %; Wangtueai and Noomhorm 2009)). Whereas Sreeja *et al.* (2023) reported a fat content of 1.3 % in the gelatin from scales of *L. rohita*. Fat content was not significantly different between isolated fish scale gelatin and commercial bovine bone gelatin. However, this value is higher than those reported in the literature, which indicates that fat contents are not completely removed during pretreatment. When it comes to the gelatin from the skin of tuna, fat content was higher (18.3 %) than Shark and Rohu (Shyni *et al.* 2014) and very higher than we reported in this study. All the results together signify the source and species from which gelatin was extracted (Table 1).

Fiber analysis

The fibre content of isolated fish gelatin in our study was 0.44 %, while the fibre content of commercial bovine bone gelatin was 0.65 % (Table 1). A bit less fiber was in the gelatin we extracted, maybe due to its purity and the fact that commercial gelatin is optimized. The gelatin we extracted is derived from collagen in the scales of fish, a protein found in animal connective tissues that does not naturally contain significant amounts of fiber. And those additional ingredients or additives would have increased the fibre in the commercial gelatin for specific purposes. This study further recommends the optimization of the extraction and characterization process and the need-based addition of fibre to the gelatin extracted from fish scales for various purposes such as gel formation, better texture and mouthfeel, water binding capacity, and increased nutritional value.

pH Analysis

The pH of isolated fish scale gelatin and commercial bovine bone gelatin was 5.67 and 6.7, respectively (Table 1), which is different from those found in the gelatin from Red Tilapia (3.05) and Black Tilapia (3.91) (Jamilah and Harvinder 2002). The pH of the gelatin from sea bass scales was 5.33, and that of grey mullet scales was reported as 5.41 (Cao *et al.* 2017). The differences in the pH values probably reflect the differences in pretreatments (including the neutralization step) used during the extraction

which involved both alkaline and acid treatments (Shyni *et al.* 2014).

Table 1. Comparison of isolated fish scale gelatine and commercial bovine bone gelatine.

Factors	Isolated fish scale gelatin	Commercial bovine bone gelatin
Moisture	11.12 %	9.02 %
Ash	2.17 %	2.36 %
Protein	82.78 %	94.27 %
Fats	1.21 %	1.19 %
Fibers	0.44 %	0.65 %
pH	5.67	6.7

Conclusion

Fish wastes, if not properly utilized, can cause ecological and hygienic problems. Reusing these materials would not only address an environmental issue but could also provide useful substances for improving human health and well-being. In the present study, gelatin from the scales of *L. rohita* was successfully extracted and compared with commercial bovine bone gelatin. The scales of *L. rohita* are found to be a sustainable and renewable source of gelatin with desirable functionalities, and it is the best alternative for mammalian gelatin in food and other industries, which is an important attribute according to the application of gelatin. The gelatin obtained from *L. rohita* may provide a low-cost, waste-free alternative to other animal sources. It may also be a useful novel biomaterial, radical scavenger, and functional food for humans.

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Conflicts of Interest

The authors declare that they have no conflict of interest.

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