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ISOLATION AND CHARACTERISATION OF COLLAGEN FROM THE WASTE MATERIAL OF *TWO IMPORTNT FRESHWATER FISH SPECIES*

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ABSTRACT

The study was designed to isolate and characterize the collagen from skin, scales and fins of *Cyprinus carpio* and *Hypophthalmichthys molitrix*. For this purpose nine fishes of each species of three weight groups were collected and categorized as W_1 (250-500 g), W_2 (501-750 g) and W_3 (751- 1000 g). The maximum skin protein content was recorded as 28.13% and 29.13% in *C. carpio* and *H. molitrix*, respectively, from the W_3. The scales of the W_3 displayed a maximum protein contents as 29.91 and 28.38% for *C. carpio* and *H. molitrix*, respectively. The maximum protein contents (19.19% and 21.37% for *C. carpio* and *H. molitrix*, respectively) were observed in the W_3 group. Protein characterisation using SDS-PAGE revealed the molecular weights (kDa) of the *C. carpio* skin, scales, and fins ranged from 126 kDa to 220 kDa, 78 kDa to 211 kDa, and 72 kDa to 141 kDa, respectively. The size of the collagen proteins of the *H. molitrix* skin, scales and fins ranged from 117 kDa to 208 kDa, 81 kDa to 219 kDa, and 72 kDa to 141 kDa, respectively. Glycine was the most abundant amino acid, whereas, Tryptophan was totally absent in all selected tissues. Significant differences were observed for amino acids within the weight groups and between the two fish species.

Key words: Fish; Proximate composition; weight groups; Amino acids; SDS-PAGE.

INTRODUCTION

A high quantity of skin, scales, and fins per unit mass has been observed in freshwater fishes; these properties confer protection in shallow waters. These abundant fish by-products may be processed to extract products such as collagen, which may increase the economic value of the fish (Thorpe *et al*., 2008). Fish farming has gained popularity due to several favourable traits, such as culture suitability in captive conditions and favourable growth in ponds. Several processing companies have been established to meet the demands of the rapid fishery development fisheries. Approximately 170,000 tons of fish-processing by-product is processed to maximise financial gains. Producers can increase revenue and decrease environmental pollution by processing these value-added products (Mirza and Bhatti, 1995).

Most fish is consumed as sliced, raw, fresh sashimi that is prepared by removing the skin, bones, and fins; these waste products are produced in great quantities by fish shops and fish-processing factories. Although fish fins are also eaten as a fried food (karaage), they may still be disposed as waste, producing pollution and emitting an offensive odour. Although the nutritional values of fish skin, bones, and fins are high, most of these useful resources are typically wasted with the exception of those used in fish meal manufacturing (Nagai and Suzuki, 2000). The increasing human population has generated an increased demand for fish consumption. This demand has resulted in increased fish catching and processing, thereby generating a significant amount of waste, such as fish skin, scales, and fins. After processing the maximum amount of edible products, the waste is typically discarded as a low-value by-product in the form of fishmeal; however, other waste uses are being exploited on a small scale (Colom, 2007). The fish processing industry utilises 60-75% of an average fish for consumable meat. An estimated 1.7 million metric tons of fish waste are generated per year in Asia alone (Brian and Hollister, 2008). Approximately 20 million tons (25%) of fish by-product are discarded annually worldwide. Although fish waste is used to produce fishmeal, fish silage may represent a feasible alternative for this waste. By-products from the fishing industry may be used as protein supplements in aquaculture feeds. The nutritional value of a balanced diet depends on the essential amino acid composition and the amino acid ratios. Vegetable protein sources are often deficient in several essential amino acids. However, the composition of these ingredients may be improved by adding protein-rich products, such as fish meal, silages, or hydrolysate (Disney *et al*., 2007).

Fish skin, scales, and fins are composed of a highly ordered three-dimensional structure of the extracellular matrix, predominantly consisting of a type I collagen fibres and calcium-deficient hydroxyapatite (Zwarts *et al*., 2006). Heat can sufficiently denature collagen into gelatine, which is primarily included in foods such as flavoured gelatine desserts. In addition to food, gelatine is used in the pharmaceutical, cosmetic,

and photography industries. In pharmaceutical applications, the collagen can be used for production of wound dressings, vitreous implants and as carriers for drug delivery. In addition, collagen has been used to produce edible casings for the meat processing industries (sausages/salami/snack sticks). Nutritionally, collagen and gelatine are poor sources of protein because they lack some of the essential amino acids in the proportions required by the human body. Collagen is used as a healing aid for burn patients, for bone reconstruction and for a wide variety of dental, orthopedic and cosmetic surgical purposes. The applicability of collagens from various sources depends on their thermal stability, which may directly correlate with the environment and body temperature of those species (Rigby, 1968). Inherent chemical or physical collagen properties vary depending on the species from which collagen is isolated (Sadowska *et al*., 2003). Recently, aquatic organisms have become increasingly desirable alternative collagen sources (Morimora *et al*., 2003). However, the physical properties depend on the origin and treatment method of collagen (Ward and Court, 1977; Ockermann *et al*., 1988). Fish collagen can be produced from the discarded portion of the fish offal waste, such as the skin, scales, and fins, which are rich collagen sources (Dun *et al*., 2008). The skins, bones, cartilage, tendons, ligaments, blood vessels, teeth, cornea, and all other vertebrate organs harbour nineteen collagen variants, types I-XIX (Senaratne *et al*., 2006). Particularly, type I collagen can be observed in all connective tissues, such as the skin and bones. Collagen from the skin of vertebrate species, such as pigs, calves, and cows, is utilised in foods, biomedical materials, cosmetics, and industrial applications (Nagai *et al.*, 2008). We aimed to utilise the fish offal of *Cyprinus carpio* and *Hypophthalmichthys molitrix* to estimate their total protein contents and to isolate and characterise the collagen obtained from waste, which is available at a low cost from Pakistan, for commercial use.

MATERIALS AND METHODS

Experimental materials: Fifty-four fish samples of the three *C. carpio* and *H. molitrix* weight groups W_1 (250-500 g), W_2 (501-750 g) and W_3 (751-1000 g) were obtained from the Fish Seed Hatchery, Faisalabad, Pakistan. The average weights and lengths of the three *C. carpio* weight groups were 422±0.05, 700±0.13, and 925±0.03 g and 34±0.06, 40±0.24, and 44±0.23 cm for W_1, W_2 and W_3, respectively. The average *H. molitrix* weights and lengths were 410±0.23, 723.33±0.03, and 900±0.21 g and 35.66±0.03, 41.45±0.41, and 45.23±0.13 cm for W_1, W_2 and W_3, respectively (Table 1). The fish were transported to the Fisheries Research Laboratory under chilled conditions and stored at -20 °C. The fish samples (skin, scales, and fins) were lyophilised at -50ºC by using a manifold freeze drier by Christ, Alpha 1-4 LD, Germany, Lypholyzer.

Proximate analysis: The proximate composition of all three-weight groups of *C. carpio* and *H. molitrix* was assessed for the skin, scales, and fins by following Association of Official Analytical Chemists (1990).

Isolation of skin, fin, and scale collagens: The collagens were isolated using the method described by Nagai and Suzuki (1999) with modifications. All samples were prepared at ambient temperature (22-23°C) with the exception of centrifugation, which, was performed at a temperature not higher than 4 °C. Following isolation, the collagen was characterised by the method described by Laemmli (1970).

Amino acid analysis: The freeze-dried samples of extracted collagen from the skin, scales, and fins were hydrolysed in the presence of 1% phenol (v/v) in an inert atmosphere with 6 N HCl at 105 °C for 24 hours. The hydrolysates were then vacuum-dried. The hydrolysates were derivatised, dried, and diluted with sample diluents and compared against the analysed standard amino acids. The area under the peak of each amino acid in the chromatogram was calculated and compared with that of the standard and reported as the number of residues per thousand amino acid contents. Amino acids were analysed from 20 µl of sample, which was performed using the Aracus Amino Acid Analyzer (MembraPure, Germany).

Quantification/estimation of extracted protein: The amount of protein in the sample solution was assessed using the method described by Bradford (1976).

Qualitative estimation of extracted collagen by SDS-PAGE: The vertical slab gel (Scie-Plas, Model No. TV 50) in the discontinuous buffer system was used for SDS-PAGE (7.5% polyacrylamide gel concentration) to analyse the extracted collagen by following the method described by Laemmli (1970) using 7.5% polyacrylamide gel concentration. A sample of 10 micro liters was loaded into each well and electrophoresis was carried out at 15 mA in the stacking gel and raised current to 25mA voltage in resolving gel. . The molecular weights of the isolated protein subunits were determined by comparing their migration rates to the migration rates of known molecular weight markers. High molecular weight markers were used to estimate the molecular weight of isolated proteins. The markers used included myosin (200 kDa), a2-macroglobulin (170 kDa), b-galactosidase (116 kDa), transferrin (76 kDa) and glutamate dehydrogenase (53 kDa). Type I calfskin collagen was used as a standard. Quantitative analysis of protein band intensity was performed using a Model GS-700 Imaging Densitometer (Bio-Rad Laboratories, Hercules, CA,

USA) with Molecular Analyst Software version 1.4 (image analysis system).

Statistical analysis: ANOVA (one way) was performed using the Minitab version 15 software package, and the means were compared by Tukey's test. Analysis of Variance and Duncan's Multiple Range tests for were performed to analyze differences between the parameters under study (Steel *et al*., 1996).

RESULTS AND DISCUSSION

Proximate composition of the fish skin, scales, and fins: The moisture content of the *C. carpio* and *H. molitrix* skin was recorded and is presented in Table 2. The moisture content of fishes of the W_3 weight group differed significantly from those of the W_1 and W_2 groups ($P<0.05$). The comparison of the mean moisture content demonstrated non-significant differences in the skin of both fish species. The skin protein contents in the W_1, W_2, and W_3 weight groups of *C. carpio* and *H. molitrix* were 27.01±0.02, 27.90±0.08, and 28.13±0.01% and 24.41±0.22, 25.11±0.08, and 28.13±0.71%, respectively. No significant difference ($P>0.05$) in the skin protein content among the three weight groups was observed (Table 2). The total skin, fat contents of the three *C. carpio* weight groups were 1.34±0.05, 3.12±0.04, and 2.01±0.14%, respectively, and those in the three *H. molitrix* weight groups were 0.98±0.13, 1.26±0.23, and 2.01±0.16%, respectively (Table 2). The total fat contents differed significantly ($P<0.05$) for both fish species. For the three weight groups, the *C. Carpi* skin ash contents were 0.22±0.01, 1.21±0.13, and 1.11±0.23%, respectively, and those for *H. molitrix* were 0.71±0.12, 0.88±0.01, and 1.11±0.06%, respectively.

The moisture contents in the scales of *C. carpio* and *H. molitrix* were 60.21±1.24, 58.83±0.02, and 55.13±1.41% and 62.25±0.20, 57.69±0.21, and 54.75±0.02% for the W_1, W_2, and W_3 weight groups, respectively (Table 3). A significant difference ($P<0.05$) in the scale moisture contents between the three weight groups was observed. The total protein contents of the *C. carpio* scales were 25.21±0.33, 27.01±0.27, and 29.91±0.72% in the three weight groups, respectively. The total protein contents in the *H. molitrix* scales were 24.32±1.07, 26.38±0.34, and 28.38±0.27% in the three weight groups, respectively, and these contents differed significantly between each weight group (Table 3). The total fat contents of the *C. carpio* and *H. molitrix* scales were 0.73±0.02, 0.75±0.01, and 0.88±0.13% and 1.09±0.01, 0.83±0.02, and 0.87±0.01% in the W_1, W_2, and W_3 weight groups, respectively. The total ash contents in the *C. carpio* scales were 4.89±0.03, 7.96±0.08, and 8.76±0.04% in the three weight groups, respectively. The total ash contents in the *H. molitrix* scales were 6.73±0.16, 6.98±0.37, and 10.58±0.11% in the three weight groups, respectively. The ash content in W_3 differed significantly from the W_1 and W_2 contents (Table 3).

The proximate analysis of the total moisture content of *C. carpio* fins revealed 61.31±1.46, 56.12±0.98, and 54.31±1.22% in the W1, W2, and W3 groups, respectively. The total moisture contents in *H. molitrix* fins were 59.98±0.05, 57.32±0.05, and 53.27±1.05% in the W_1, W_2, and W_3 weight groups, respectively (Table 4). The moisture contents in the three weight groups differed significantly among each other ($P<0.05$). The total protein contents in the *C. carpio* and *H. molitrix* fins were 17.11±0.36, 18.66±0.19, and 19.19±0.45% and 19.23±0.01, 20.41±0.09, and 21.37±0.06% in the W_1, W_2, and W_3 weight groups, respectively. A significant difference ($P<0.05$) in the protein contents in the W_1, W_2, and W_3 weight groups was observed. The total fat contents in *C. carpio* fins were 5.75±0.08, 7.63±0.03, and 7.01±0.02% in the W_1, W_2, and W_3 weight groups, respectively (Table 4). The total fat contents in the *H. molitrix* fins were 5.33±0.03, 6.71±0.07, and 8.83±0.06% in the W_1, W_2, and W_3 weight groups, respectively. The total ash contents of the *C. carpio* and *H. molitrix* fins were 13.32±0.43, 14.52±0.20, and 15.79±0.17% and 11.20±0.31, 10.61±0.20, and 13.21±0.17% in the W_1, W_2, and W_3 weight groups, respectively. The statistical analysis revealed that the weight groups differed significantly ($P<0.05$) from each other. The maximum protein content was recorded for the *H. molitrix* weight group W_3. The two fish species displayed a non-significant difference ($P>0.05$) with respect to their protein contents. The total protein and fat contents increased with increasing weight and size. These findings were consistent with the findings of Jabeen ad Chaudhry (2011) and with those of Javed (1988) and Mahboob *et al*. (1996), who reported a positive, highly significant correlation between the total protein and fat contents in carp. Isolating collage from fish offal, such as the skin, may represent an efficient, alternative collagen source, which is similar to the with findings of Gomes- Guillen *et al*. (2002) and Shao *et al*. (2009), who reported the presence of collagen in tissues such as the skin and scales.

In the present study, a proximate analysis of the scales and fins of both fish species revealed a significant difference in the total protein contents within the three weight groups. The protein contents appeared to increase in the scales and fins with increasing fish weight. The presence of collagen in the fish scales, which are considered waste, was also supported by the findings of Zhang *et al*. (2007), who reported a high amount of collagen in fish scales and bones. These results that identify collagen from a waste source, such as fish processing by-products, have also been reported by Nagai and Suzuki (1999), who revealed that type I collagen

could be isolated from fish skin, bones, and fins. We observed the most protein content in the skin, whereas the scales and fins displayed relatively low amounts of protein. These findings were corroborated by the findings of Iqbal (2002). The variation in the proximate analysis of both fish species may be attributed to the inherent potential of each fish species. Nalinanon *et al*. (2007) reported that fish protein contents depend on the fishing period and genetic potential. When food is lacking, albumin and globulin are degraded, and the amount of collagen in the skin increases. The differences between fish species and the protein isolation method may contribute to the differences between the results.

Collagen profiling in the skin, scales, and fins by SDS-PAGE: Collagen profiling of the skin of both fish species was performed using SDS-PAGE. The gel photographs of collagen extracted from *C. carpio* skin demonstrated that a marker with known molecular weights ranging from 45 to 200 kDa purchased from Sigma-Aldrich was run in the first well. The acid-soluble collagen extracted from *C. carpio* skin was run in wells 2-4, which demonstrated three fractions with molecular weights of 220, 135, and 126 kDa for the W_1, W_2, and W_3 weight groups, respectively (Figure 1). The acid-soluble collagen extracted from *H. molitrix* skin was run in wells 2-4 of a separate gel, which revealed three fractions with molecular weights of 208, 139, and 117 kDa corresponding to the W1, W_2, and W_3 weight groups, respectively.

The acid-soluble collagen extracted from *H. molitrix* scales were run in wells 5, 6, and 7, which corresponded to the W_1, W_2, and W_3 fish weight groups, respectively, and revealed five fractions in each well with molecular weights of 219, 174, 133, 93, and 81 kDa. The gel photograph revealed no differences in the collagen extracted from the scales of different fish weight categories (Figure 2). The acid-soluble collagen extracted from *C. carpio* scales were run in wells 5, 6, and 7, corresponding to categories W_1, W_2, and W_3, respectively, and revealed five fractions in each well with molecular weights of 211, 165, 130, 91, and 78 kDa. The *C. carpio* fin collagen was run in wells 8, 9, and 10, corresponding to groups W_1, W_2, and W_3, respectively, and revealed three factions in each well with molecular weights of 141, 109, and 72 kDa (Figure 1). The extracted collagen from *H. molitrix* fins was run in wells 8, 9, and 10, corresponding to the W_1, W_2, and W_3 fish weight groups, respectively, and revealed three factions in each well with molecular weights of 141, 102, and 72 kDa. The gel photograph demonstrated that no difference in the fin collagen profiles extracted from three categories of fish (Figure 2).

There were no differences in the skin collagen profiles among the three weight categories when characterised by SDS-PAGE (Figure 2). These findings are consistent with the findings of Duan *et al*. (2009), who reported that skin collagen from fish of various ages was readily soluble in dilute acetic acid and was characterised using SDS-PAGE. This characterisation is also consistent with the findings of Nomura *et al*. (1996), who adopted the same method. In the present study, three distinct bands of molecular weights ranging from 126 to 220 kDa in *C. carpio* and from 117 to 208 kDa in *H. molitrix* were observed; these findings were similar to those reported by Ogawa *et al*. (2003) and Yung *et al*. (2005). The isolated collagen appears to be type I, which was previously observed by Kimura *et al*. (1993) and Muyonga *et al*. (2004), who reported that the carp skin, scale, and bone collagen was the type I based on its electrophoretic mobility.

The gel photograph demonstrated that there were no differences in the collagens extracted from the scales of the different fish groups (Figure 1). These results are consistent with the findings of Ogawa *et al*. (2003) and Zhang *et al*. (2007), who also reported a lack of a significant difference between the subunit molecular masses of the collagen from fins and scales. The authors did not observe a difference in the mobility of collagen α-chains between carp fish collagen, indicating that the collagens may possess extremely similar molecular masses. Nagai and Suzuki (2000); Nagai *et al*. (2004); Rodziewicz-Motowidlo *et al*. (2007) and Nagai *et al*. (2008) reported that collagen is the primary protein in preparing 75% of the total protein contents. The remaining 25% is composed of smaller fragments that appear under the α bands corresponding to the molecular weight ranging from 70-150 kDa, including the probable elastin bands (8%) and the remaining unidentified proteins and peptides (17%).

Amino acid analysis: Amino acids were profiled from *C. carpio* and *H. molitrix* skin, scales, and fins from three weight groups (Table 5-7). The significant differences remained significant for a skin amino acid profile for both fish species in the three weight groups (Table 5-7). Few amino acids displayed non-significant differences. Overall, glycine was the most abundant amino acid identified in the skin, scales, and fins of both fish species. The hydroxyproline and proline contents of collagen from the silver carp skin are 19.2 percent, which is quite similar to that of collagen from common carp skin (which has 190 residues/1000 residues). The results suggest that the two-collagen types may possess similar thermal stabilities due to analogous living conditions. The hydroxyproline and proline contents in *C. carpio* and *H. molitrix* were extremely similar.

Duan *et al*. (2009) reported that glycine is a major amino acid in carp collagen. The results of the

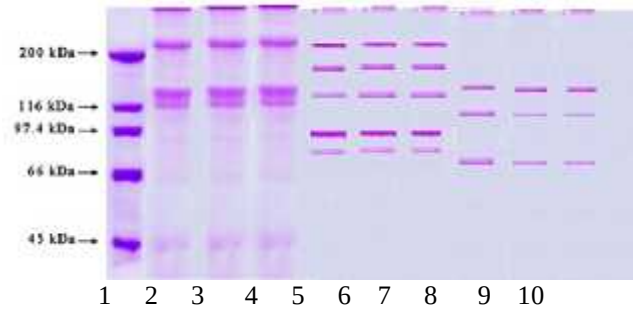

Figure 1: Gel photograph demonstrating the protein molecular weight marker (well 1) and the collagen patterns from the skin (wells 2, 3, and 4), scales (wells 5, 6, and 7) and fins (wells 8, 9, and 10) of *Cyprinus carpio* from three weight groups (W_1, W_2, and W_3, respectively).

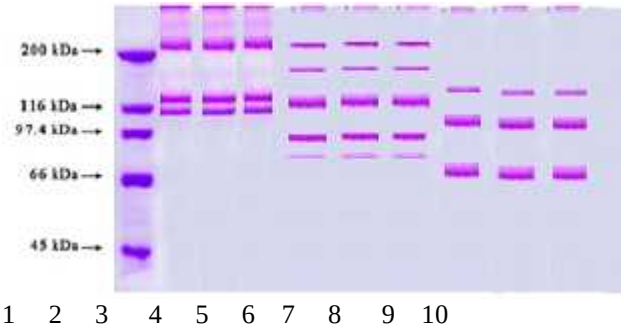

Figure 2: Gel photograph demonstrating the protein molecular weight marker (well 1) and the collagen patterns from the skin (wells 2, 3, and 4), scales (wells 5, 6, and 7) and fins (wells 8, 9, and 10) of *Hypophthalmichthys molitrix* from three weight groups (W_1, W_2, and W_3, respectively).

present study are also supported by the findings of Zhang *et al.* (2007) and Kui and Mendis (2008), who demonstrated that glycine is the major amino acid in each collagen type. Collagen contents are closely correlated with thermo stability, as reported by Shao *et al*. (2009). The present study suggests that *H. molitrix* collagen exhibits a higher thermal stability compared to *C. carpio*. However, it is lower than mammalian collagen, and these findings are consistent with the observations of Rodziewicz-Motowidlo *et al*. (2007), who reported that the hydroxyproline and proline contents of silver carp are slightly higher than those of a carp. The amino acid contents of both fish types vary among the three tissues selected (skin, scales, and fins). Similar results have been reported by Nagai *et al*. (2004), who observed that the collagen amino acid contents correlated with the water temperature of their normal habitat. Methionine and cysteine were negligible in the *C. carpio* and *H. molitrix* skin, scales, and fins from the three weight groups. Collagen from the outer *H. molitrix* skin is typically considered to be type I collagen. Similar findings have also been reported by Rodziewicz-Motowidlo *et al*. (2007), who demonstrated that *H. the molitrix* skin harbours extremely low methionine content; the authors also considered collagen from silver carp skin as type I. Tryptophan was not observed in the collagen isolated from the skin, scales, and fins of both fish species. Tyrosine and phenylalanine were identified in extremely small quantities, which may be attributed to the presence of type I collagen. Similar findings have also been reported by Chen *et al*. (2004).

Table 1. Weight and length parameters of *Cyprinus carpio* and *H. molitrix*.

Category	*Cyprinus carpio*		*H. molitrix*	
	Weight (g)	Length (cm)	Weight (g)	Length (cm)
W_1	422±0.05	34±0.06	410±0.23	35.66±0.03
W_2	700±0.13	40±0.24	723±0.03	41.45±0.41
W_3	925±0.03	44±0.23	900±0.21	45.23±0.13

Table 2. Proximate analysis of *Cyprinus carpio* and *Hypophthalmichthys molitrix* skin from three weight groups

Species	Weight Category	Moisture (%)	Protein (%)	Fat (%)	Ash (%)	Carbohydrate (%)
C. carpio	W_1	69.29±0.04^a	27.01±0.02^a	1.34±0.05^a	0.22±0.11^a	2.59±0.39^a
	W_2	70.45±0.02^a	27.90±0.08^a	3.12±0.04^b	1.21±0.13^b	1.75±0.17^b
	W_3	68.18±0.03^a	28.13±0.01^a	2.01±0.14^c	1.11±0.23^b	2.89±0.16^a
H. molitrix	W_1	72.39±0.51^a	24.41±0.22^a	0.98±0.13^a	0.71±0.12^a	2.09±0.09^a
	W_2	71.51±1.07^a	25.11±0.08^a	1.26±0.23^b	0.88±0.11^a	4.69±0.45^b
	W_3	68.18±0.87^b	28.13±0.71^b	2.01±0.16^c	1.11±0.06^b	2.89±0.08^a

Means in the same column for each species followed by different letters are significantly different according to Ducan's test ($P \leq 0.05$)

Table 3. Proximate analysis of *Cyprinus carpio* and *Hypophthalmichthys molitrix* scales from three weight groups.

Species	Weight Category	Moisture (%)	Protein (%)	Fat (%)	Ash (%)	Carbohydrate (%)
C. carpio	W_1	60.21±1.24[a]	25.21±0.33[a]	0.73±0.02[a]	4.89±0.03[a]	7.22±0.07[a]
	W_2	58.83±1.10[b]	27.01±0.27[b]	0.75±0.01[a]	7.96±0.08[b]	6.03±0.03[b]
	W_3	55.13±1.41[c]	29.91±0.72[c]	0.88±0.03[b]	8.76±0.04[c]	6.06±0.09[b]
H. molitrix	W_1	62.25±0.20[a]	24.32±1.07[a]	1.09±0.01[a]	6.73±0.16[a]	5.37±0.23[a]
	W_2	57.69±0.21[b]	26.38±0.34[b]	0.83±0.02[b]	6.98±0.37[a]	8.32±0.43[b]
	W_3	54.75±0.02[c]	28.38±0.27[c]	0.87±0.01[b]	10.58±0.1[b]	5.12±0.44[b]

Means in the same column for each species followed by different letters are significantly different according to Ducan's test ($P \leq 0.05$)

Table 4. Proximate composition of *Cyprinus carpio* and *Hypophthalmichthys molitrix* fins from the three weight groups.

Species	Weight Category	Moisture (%)	Protein (%)	Fat (%)	Ash (%)	Carbohydrate (%)
C. carpio	W_1	61.31±1.46[a]	17.11±0.36[a]	5.75±0.08[a]	13.32±0.43[a]	4.51±0.14[a]
	W_2	56.12±0.98[b]	18.66±0.19[b]	7.63±0.03[b]	14.52±0.20[b]	6.12±0.05[b]
	W_3	54.31±1.22[c]	19.19±0.45[c]	7.01±0.02[b]	15.79±0.17[c]	4.68±0.03[a]
H. molitrix	W_1	59.98±0.05[a]	19.23±0.01[a]	5.33±0.03[a]	11.20±0.31[a]	5.53±0.07[a]
	W_2	57.32±0.05[b]	20.41±0.09[b]	6.71±0.07[b]	10.61±0.20[b]	5.47±0.23[a]
	W_3	53.27±1.05[c]	21.37±0.06[c]	8.83±0.06[c]	13.21±0.17[c]	7.23±0.06[b]

Means in the same column for each species followed by different letters are significantly different according to Ducan's test ($P \leq 0.05$).

Table 5. Amino acid composition of collagen isolated from skin of *Cyprinus carpio and Hypophthalmichthys molitrix* from three weight groups

Amino Acid	*H. molitrix*			*Cyprinus carpio*			SE and Main effects		
	1st	***2nd***	***3rd***	***1st***	***2nd***	***3rd***	**Sp**	W	Sp X W
Aspartic acid	48	48	46	46	45	46	0.14***	0.24	0.24**
Threonine	18	18	17	20	21	21	0.19***	0.33*	0.33
Serine	30	28	32	31	30	29	0.19*	0.33	0.33
Glutamic acid	71	73	75	69	69	67	0.19***	0.33	0.33**
Glycine	260	254	250	276	270	273	0.16***	0.27**	0.27***
Alanine	81	83	81	89	90	91	0.24*	0.41	0.41*
Valine	26	19	21	19	17	17	0.19**	0.33	0.33
Methionine	14	16	17	13	11	13	0.24***	0.41*	0.41*
Isoleucine	11	12	15	6	7	7	0.22***	0.37	0.37
Leucine	21	21	23	19	17	19	0.24***	0.41	0.41*
Tyrosine	4	5	5	3	2	3	0.14***	0.24	0.24*
Phenylalanine	15	13	14	12	13	11	0.16	0.27*	0.27
Hydrolysine	6	7	7	5	6	5	0.17**	0.29*	0.29
Lysine	26	27	29	25	28	23	0.22*	0.37**	0.37
Histidine	4	5	4	4	3	4	0.14**	0.24	0.24**
Arginine	58	57	54	50	53	56	0.53	0.92*	0.92
Hydroxyproline	87	87	80	69	72	76	0.16***	0.27*	0.27
Proline	87	85	83	96	96	94	0.16**	0.27**	0.27
Imino acid	133	142	147	148	150	145	0.22***	0.38***	0.38***

1st= weight group; 2nd = weight group 2; 3rd = weight group 3; w =Weight; Sp× w= Species× Weight interaction

Table 6. Amino acid composition of collagen isolated from scales of *Cyprinus carpio and Hypophthalmichthys molitrix* from three weight grous.

Amino Acid	*H. molitrix*			*Cyprinus carpio*			SE and Main effects		
	1st	*2nd*	*3rd*	*1st*	*2nd*	*3rd*	Sp	W	Sp X W
Aspartic acid	38	36	39	37	38	36	0.16***	0.27	0.27***
Threonine	27	23	23	21	23	24	0.16***	0.27***	0.27**
Serine	33	34	35	34	34	36	0.14***	0.24***	0.24
Glutamic acid	65	66	67	66	67	67	0.18***	0.30***	0.30
Glycine	284	285	289	281	279	286	0.47***	0.80***	0.80***
Alanine	95	98	93	94	96	91	0.51***	0.88***	0.88***
Valine	20	18	22	19	19	18	0.12*	0.22	0.22**
Methionine	12	15	16	13	12	13	0.11***	0.19*	0.19**
Isoleucine	10	12	11	10	11	12	0.14	0.24**	0.24*
Leucine	19	18	14	16	15	18	0.20	0.35***	0.35
Threonine	1	2	1	3	4	5	0.14***	0.24*	0.24**
Phenylalanine	9	12	15	10	8	9	0.14*	0.24***	0.24
Hydrolysine	8	8	8	8	7	7	0.14***	0.24*	0.24*
Lysine	25	24	26	26	23	23	0.14	0.24***	0.24*
Histidine	6	6	4	4	3	4	0.14***	0.24	0.24*
Arginine	44	42	38	46	46	43	0.30*	0.53**	0.53
Hydroxyproline	78	79	77	80	82	77	0.16***	0.27**	0.27***
Proline	90	91	93	95	91	92	0.23	0.40***	0.40**
Imino acid	136	131	129	137	142	139	0.36	0.63**	0.63*

1st= weight group; 2nd = weight group 2; 3rd = weight group 3; w =Weight; Sp× w= Species× Weight interaction

Table 7. Amino acid composition of collagen isolated from fins of *Cyprinus carpio* and *H. molitrix* from three weight groups.

Amino Acid	*H. molitrix*			*Cyprinus carpio*			SE and Main effects		
	1st	*2nd*	*3rd*	*1st*	*2nd*	*3rd*	*sp*	*w*	*sp×w*
Aspartic acid	41	43	41	37	36	37	0.23***	0.40*	0.40
Threonine	32	31	34	28	29	29	0.19***	0.33**	0.33
Serine	40	40	42	44	45	47	0.22***	0.37**	0.37
Glutamic acid	79	78	78	71	72	75	0.22***	0.37	0.37**
Glycine	252	254	252	265	264	262	0.22	0.37	0.37*
Alanine	76	74	74	85	87	86	0.22*	0.37**	0.37***
Valine	21	22	21	15	14	17	0.24***	0.41	0.41*
Methionine	15	15	15	17	18	18	0.19***	0.33	0.33*
Isoleucine	11	11	10	7	8	8	0.19***	0.33	0.33
Leucine	22	24	22	23	22	23	0.22***	0.37*	0.37
Tyreonine	5	6	6	7	8	7	0.17***	0.29**	0.29*
Phenylalanine	12	11	9	12	12	14	0.17***	0.29	0.29***
Hydrolysine	10	8	11	8	9	8	0.19*	0.33**	0.33
Lysine	26	25	24	23	22	22	0.24**	0.41*	0.41
Histidine	11	12	11	8	9	8	0.14***	0.24*	0.24
Arginine	65	66	67	54	53	53	0.24	0.41	0.41
Hydroxyproline	80	80	82	76	75	72	0.24***	0.41	0.41**
Proline	76	75	76	88	87	85	0.28***	0.49	0.49
Imino acid	126	125	125	132	130	129	0.24	0.41*	0.41**

1st= weight group 1; 2nd = weight group 2; 3rd = weight group 3; w =Weight; Sp× w= Species× Weight interaction

Conclusions: An adequate amount of collagen in the skin, scales, and fins of *C. carpio* and *H. molitrix* can be easily isolated and utilised for various industrial purposes, such as cosmetics and pharmaceutical industries, at a low or no cost. The proximate analysis indicated that the nutritional values of fish skin, scales, and fins are high, and these useful resources may be wasted with the exception of their occasional use in fish

meal manufacturing. Glycine was the most abundant amino acid in all of the tissues selected, including the skin, scales, and fins, whereas tryptophan was completely absent from all of the selected tissues.

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