Purification and Characterisation of Collagen of *Chanos chanos*

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Collagen extracted individually from the muscle, skin and waste comprising bones and viscera of *Chanos chanos* was partially purified and fractionated to acetic acid soluble collagen (ASC) and pepsin digestible collagen (PDC). Fish waste had only ASC while skin and muscle had small amounts of PDC in addition to ASC. The yield of partially purified ASC and PDC were 0.38 and 0.09% respectively from muscle and 20.14 and 5.6% from skin. Yield of ASC from waste was 0.2%. Total nitrogen, amino acid composition, electrophoretic pattern and approximate molecular weight of the partially purified collagens are reported.

Key words: Collagen, *Chanos chanos*, purification, characterisation

Collagen is an extracellular matrix protein playing an important role as connective tissue. In the white muscle of carp it accounts to 3% of the total protein. (Kimura *et. al.*, 1979). In fish, collagen is mostly concentrated in skin, fins and skeleton (Sikorski *et. al.*, 1984). The myocommata, the membrane surrounding the myotomes of the muscle fibres, is made up of collagen. Hence, it is reported to play a major role in the phenomenon of 'gaping' (Love and Huq, 1970). The content of collagen in fish varies from fish to fish and also with season.

Characterisation of fish collagen has been studied in detail (Montero *et. al.*, 1990; Kimura *et. al.*, 1981; Sato *et. al.*, 1986; Lewis and Piez, 1964; Kimura and Tanaka, 1986). Literature on collagen in Indian fishes is scanty. Results of a study to characterise the collagen in muscle, skin and, waste comprising bones and viscera of *Chanos chanos* are reported in this paper.

Materials and Methods

Farmed brackish water fish, *Chanos chanos* was used. Immediately after harvest the fish was iced. Skin, muscle and waste (mainly bones and viscera) were taken for extraction of collagen. All operations were carried out at 5°C. The extraction procedure of Montero *et. al.* (1990) with slight modification was followed.

The source material was minced and mixed with 30 volumes of 0.1N sodium hydroxide and kept stirred for 24 h over a magnetic stirrer. The treated mass was strained through a coarse sieve. The process was repeated twice and the residue was washed twice with 30 volumes of chilled distilled water. The residue was homogenised in a Polytron homogeniser with 30 volumes of 0.5M acetic acid for one min. and

the same was stirred over a magnetic stirrer for 24 h. The supernatant after centrifugation (35000 x g, 1h) was collected. The residue was once again extracted with acid as above and the combined supernatant was taken as acid soluble collagen (ASC).

The residue from the previous step was homogenised with 30 volumes of 0.5M formic acid for 1 min and stirred for 24 h. A solution of pepsin (enzyme/tissue ratio 1:100) was added to this and kept stirring for another 24 h. The supernatant after centrifuging was taken as pepsin digestible collagen (PDC).

Crystalline sodium chloride was added to both supernatants to the level of 10% and stirred for 24 h to precipitate the collagen. The precipitate was suspended in Tris-glycine buffer (50 mM containing 0.2M NaCl, pH 7.4) and dialysed against the same buffer for 24 h, centrifuged as before and the precipitate was frozen immediately until used for analysis.

The samples were analysed for moisture, total nitrogen and nonprotein nitrogen as per AOAC (1990) methods. Collagen samples were hydrolysed in 6 N HCl at 110°C for 24 h in evacuated tubes and the hydrolysate was analysed for amino acids using Shimadzu amino acid analyser. Tryptophan was estimated after alkali hydrolysis by colorimetry (Sastry and Tammura, 1985). Electrophoresis was carried out on polyacrylamide gel containing sodium dodecyl sulphate (SDS-PAGE) as per the method of Laemmli (1970), using 6% running gel (2.6% cross linking) and 3% stacking gel. The protein bands stained by Coomassie brilliant blue were scanned at 545 nm using a gel scanner attachment to a Hitachi spectrophotometer (model - 556-0008).

Results and Discussion

Yield and total nitrogen of collagen from different parts are given in Table 1. The ASC was high in skin and very low in muscle and waste. PDC was absent in waste. All samples had some nonprotein nitrogen in the range 0.17- 0.25%.

Table 1. Yield and nitrogen content of collagen from different sources

Collagen type	Source	Yield*	Nitrogen, %
ASC	Skin	20.14	3.22
	Muscle	0.38	5.21
	waste	0.22	-
PDC	Skin	5.60	3.52
	Muscle	0.09	-
	Waste	-	-

* Expressed as g dry material per 100 g wet source material

The amino acid pattern of the collagens is given in Table 2. Glycine, alanine, glutamic acid and arginine constituted about 70% of the total amino acids. Glycine was the highest fraction accounting for about 38% in collagen from skin and muscle whereas it was 29% in collagen from waste. This is in agreement with the earlier reports (Sikorski *et. al.*, 1984; Montero *et. al.*, 1990; Nip *et. al.*, 1981). The

hydroxyproline and proline contents in the experimental samples were less compared to the reports in earlier studies, but the histidine content of skin was almost double that of the reported values. This may be due to the species variation or due to the habitat. The total essential amino acid contents of collagens from different sources ranged between 170 and 178 amino acid residues per 1000 residues. This compares well with the reported values in hake, code and catfish (Sikorski *et. al.*, 1984).

Table 2. Amino acid composition of collagen from different source (number of residues / 1000 amino acid residues)

Amino acid	Skin ASC	Skin PDC	Muscle ASC	Waste ASC
Hydroxy proline	14.92	17.66	12.84	17.85
Asparatic acid	55.92	51.91	56.82	51.60
Threonine	23.14	23.10	25.00	26.10
Serine	40.39	39.26	36.60	36.11
Glutamic acid	84.95	77.81	84.07	72.37
Proline	20.11	24.80	19.79	21.19
Glycine	383.80	391.78	378.81	287.78
Alanine	172.06	157.00	175.85	129.64
Cysteine	0.00	0.00	0.00	0.00
Valine	20.47	19.76	21.36	24.76
Methionine	9.60	7.62	11.11	1.73
Isoleucine	13.18	12.25	14.17	16.15
Leucine	22.48	23.57	23.99	34.95
Tyrosine	2.49	1.65	2.61	6.27
Phenylalanine	16.14	15.94	16.98	17.38
Histidine	15.21	21.79	7.77	7.57
Hydroxylysine	7.76	10.02	11.03	5.99
Lysine	26.89	30.12	26.92	26.08
Tryptophan	0.00	0.00	0.00	0.00
Arginine	59.07	61.50	60.70	46.26

The electrophorogram of SDS-PAGE of the samples are given in Fig 1. The acid soluble fraction from muscle, skin and waste showed six peaks. Electrophorogram of collagen from muscle skin and waste showed a band at the origin with an approximate molecular weight of 2,00,000. Montero *et. al.* (1990) reported higher cross linking in collagen of connective tissue from hake and trout muscle and inferred that it was due to γ collagen chains. The high molecular bands observed in the present study could be due to γ collagen chain. The other five bands with molecular weights below 2,00,000 may include α and β chains of collagen (Yoshinaka *et. al.*, 1976). Asghar and Henrickson (1982) reported high proportions of α monomers and β dimers and a small quantity of γ trimers in collagen in its acid soluble fraction. Hence the high molecular band could be the γ chains and the low molecular bands as α and β chains. Niyibizi *et. al.* (1984) reported further classification of chains of collagen as α_1 and β_2 based on electrophorogram of the collagen from human source under identical conditions. Kimura *et. al.* (1981) identified some highly cross linked material in the collagen from lobster skin as β and γ chains with high molecular weights. This also

supports the earlier statement on the presence of β and γ chains in the samples. The other bands in the electrophorogram appear to be due to the presence of non collagen impurities in the partially purified collagen.

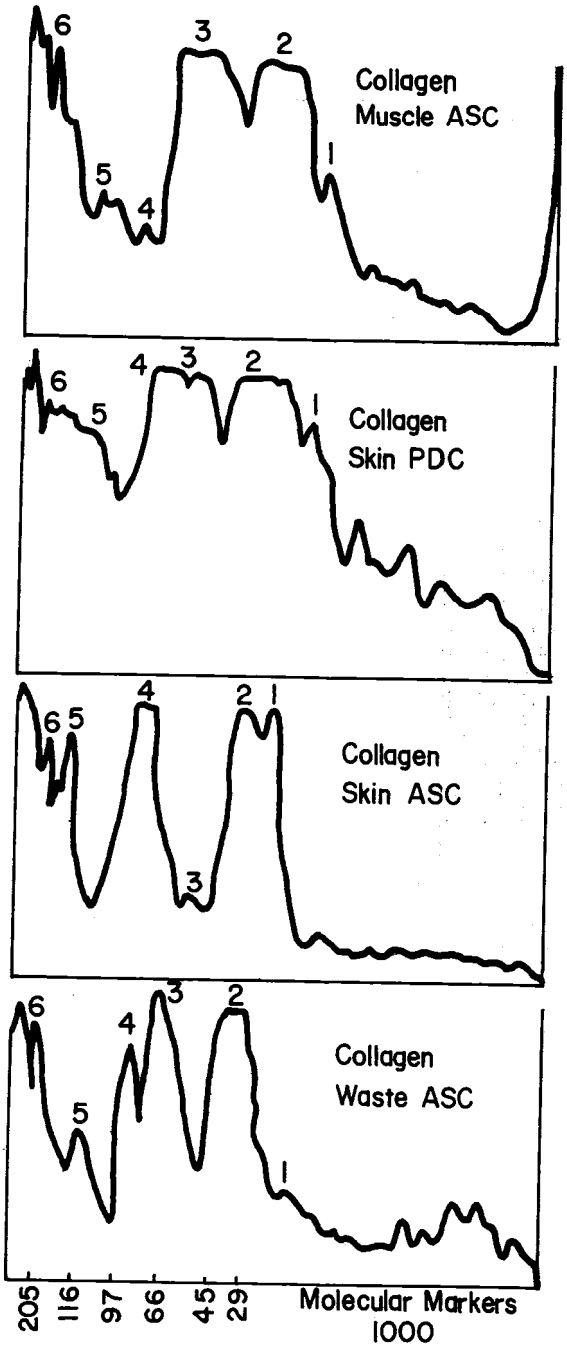

Fig. 1. Densitometric scan of partially purified collagen from *Chanos chanos*

The results suggest that the fish skin is a rich source of acid soluble and pepsin digestible collagens. Collagen of muscle and waste is rich in ASC compared to PDC. There is difference in the sub units with respect to the source.

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