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Fish Processing Waste: A Promising Source of Type-I Collagen

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Abstract

Collagen is the most abundant protein of the animal kingdom. It has varied application in different fields such as pharmaceutical, biomedical, cosmeceutical, etc. Among the twenty nine different types of collagen identified till date, type I is the most prominent one. Most of the marketed collagen is obtained mainly from the animal source which includes bovine and porcine skin and bones, chicken waste, etc. But due to its high cost and the onset of diseases such as FMD(Foot-and-Mouth Disease), BSE (Bovine spongiform encephalopathy) and TSE, cheaper and safer sources of collagen have been probed for. Thus the role of marine source came in which has been acknowledged as a promising alternative collagen source owing to the advantages such as absence of zoonosis risks, lack of dietary constraint, easy availability and higher yields.

Fish processing waste is a major environmental hazard. It includes both the solid and liquid waste. The solid waste, which includes the remains of fish such as its head, skin, scales, fins, etc, discarded after the step of processing poses the foremost threat to the environment. Presently these offals are made use of as a source of value added products such as proteins, minerals, gelatin, bioactive peptides, fish oils, enzymes, biogas/biodiesel, amino acids, collagen, etc. Use of this waste for collagen production would not only save our environment, but also be the cheapest source of raw material. It is a potential source of collagen which has been proved from the research work carried out utilising the same. Thus, this review article summarises the work carried out to obtain type I collagen from the wastage of different fishes.

Keywords: Type-I Collagen, Fish waste, ASC, PSC, Marine source.

Introduction

Collagen is a structural fibrous protein found abundantly in animals accounting for nearly 30% of the total protein present in their body. It is mainly present in extracellular matrix of various connective tissues such as the skin, cartilage, blood vessel, bone, teeth, ligament, tendon, etc. (1). It plays a crucial role in maintaining the structure of different tissues and also helps in tissue remodeling, adhesion, etc. Collagen is mainly obtained from porcine and bovine source. But due to its high cost, onset of diseases (such as FMD, BSE and TSE) and religious hindrances, cheaper and safer sources of collagen have been probed for. Thus the role of marine organisms came in which has been acknowledged as a promising alternative collagen source owing to their advantages which includes absence of zoonosis risks and religion based dietary constraint, easy availability and higher yields, etc. They are abundantly present in the vertebrates as well as invertebrates. In case of vertebrates, collagen forms the structural element of skin, bone, cartilage, etc; whereas in the invertebrates, they are present in their body walls, cuticles, etc.

Collagen is a naturally occuring protein. The collagen molecule is made up of three polypeptide chains (á chains). These chains may be identical or two or three of them may be different.Each chain exhibits a left handed helix conformation. The three chains are twisted to form a triple helix which is stabilised by the presence of hydrogen bond. The molecule exhibits a distinct tertiary structure due to its characteristic repetitive sequence of triplet Gly-X-Y with each chain being more than 1000 residues long. Collagen types differ based on the different X and Y ie the amino acids linked to glycine. Mostly, X and Y are the imino acids proline and hydroxyproline.

Presently there are 29 different collagen types available. Fibril forming collagen represents almost 90% of the collagen present in our body. It includes collagen type I,II,III,V, XI, XXIV, AND XXVII (2,3). Among these type I collagen is the most abundant one. Its abundance is an outcome of its extensive occurrence in almost all connective tissues present in the body except hyaline cartilage (4,5). It comprises nearly 90% of the organic bone mass. It is prominently found in the skin, tendons, ligament, cornea, ligature, etc. (6). The triple helix of type I collagen is a heterotrimer with two identical á 1(I)-chains and one á 2(I)-chain. á 2 chain which is hydrophobic in nature, stabilises the collagen (7).

Collagen has diverse application in various fields. It is used mainly in the biomedical, pharmaceutical and medical industry. Its applicability as a biomaterial in drug delivery systems and in tissue engineering is based on the unique characteristics such as high biodegradability, biocompatibility, cell attachment capability, high tensile strength, weak antigenecity, abundance, easily purifiable, etc. (8-13). It is also used in the food, cosmeceutical, film and leather industries etc. (14-16).

Currently type-I collagen is considered as the gold standard in the field of tissue engineering (17) collagen based biomaterials have prominent application especially the collagen scaffold. It is utilised for the study of cell behaviour, as nervous system models, testing of anti-cancer drugs to cultivate ex-vivo organs, as 3-D model for bone diseases, etc. (18-29). Type-I collagen is obtained industrially from bovine achilles tendon. Due to its soaring cost, cheaper sources of collagen are on the look out. Thus came in the role of fish processing waste as a source of collagen. Collagen obtained from the wastage of fishes have been utilized in diverse fields. A few examples of the same obtained from the processing waste are given below:

- The collagen from the swim bladder of marine cat fish (Tachysurus maculatus) has been used in the medical field as a wound healing matrix in the form of collagen-chitosan sheet.
- Lates calcarifer scales have been used as dressing material for wounds in the form of collagen sheet.
- Collagen type I from the outer skin of marine eel fish (Evenchelys macrura) is used as drug delivery system.
- Collagen from swim bladder of Bester sturgeon fish in the form of hydrogel has potential to be used as biomaterial in the field of tissue engineering.
- Collagen based biomaterial using swim bladder matrix of Rohu (Labeo rohita).

Fish processing waste

In the current scenario, fish processing waste poses a major threat to our environment by causing pollution. The wastes are both in solid and liquid form (30). The solid waste consists of tail, head, skin, scales, fin , gut, etc. It accounts for nearly 75% of the total weight of fish, (even after filleting) out of which the skin and bones makes up 30%. These offals can be made use of as a source of value added products such as proteins, minerals, gelatin, bioactive peptides, fish oils, enzymes, biogas/biodiesel, amino acids, collagen,etc (31). The usage of FPW increases the economic value of the fish also and helps to increase the income. In this review we have focused on summarising about type I collagen extracted from these discards, which has not been done so far.

Skin : Fish skin accounts for nearly 6% of the live weight of fish especially in carps. It forms a major portion of the processing waste and is easily obtainable and abundant. It is considered a significant source of highly soluble collagen (32). The main component of skin is type I collagen. Due to the above reasons, majority of the extraction of type I collagen has been carried out using fish skin. Usually fish skin is used as soil fertilizer or animal food supplement. If it is used to obtain collagen, it will definitely increase the demand of the waste.

Collagen type-I has also been extracted from the skin of Striped catfish (Pangasianodon hypophthalmus), rock fish (Sebastes schlegeli), marine eel fish (Evenchelys macrura), silver-line grunt, albacore tuna, brown backed toadfish, baltic cod, nile perch (young and adult), cat fish (Tachysurus maculatus),Catla catla, longtailtuna Thunnustonggol, Cirrhinus mrigala ,Alaska pollack, etc. (33-38). Nileperch, Leather jacket and Japanese sea-bass skin have yielded the highest amount of collagen compared to the other fish skins. When comparing the denaturation temperature Marine eel fish and Niletilapia have values close to mammalian range. Collagen type I obtained from the skin of different fishes have been included in table 1.

Bones : Bones are separated after the muscle proteins are removed from the fish frames. It contains nearly 30% of the collagen in all. The minerals present in them are calcium, phosphorous, hydroxyapatite, etc (31). Type I collagen can also be obtained from the bones of Leather jacket (Odonus niger), etc. Ayu has the highest yield of collagen compared to the other fish bone extracts. Collagen type I obtained from the bones of different fishes have been included in table 2.

Scales : Collagen type-I was also obtained from black drum, sheep's head sea bream, red tilapia, skipjack tuna, ayu, yellow sea bream and horse mackerel, pagrus major, Catla catla, Cirrhinus mrigala, lates calcarifer, Pacific saury (Cololabis saira), lizard fish (Saurida spp.) and horse mackerel (Trachurus japonicus) from Japan and Vietnam and grey mullet (Mugil cephalis), flying fish (Cypselurus melanurus) and yellowback seabream (Dentex tumifrons) from Japan, threadfin bream (Nemipterus japonicas), etc. (55-59). Sardine scales yielded the highest amount of collagen. Denaturation temperature (Td) of Grass carp was closest to mammalian value.

Collagen type I obtained from the scales of different fishes have been included in table 3.

Fins : Collagen type-I is extracted from the fins of different fishes such as Catla catla, Cirrhinus mrigala, Japanese seabass, longtailtuna Thunnustonggol, Tilapia, threadfin bream (Nemipterus japonicas) ,etc (63,64). The caudal fin of Japanese seabass was used to extract collagen which yielded higher acid insoluble collagen(36.4% dwb) when compared to ASC(5.2% dwb). The denaturation temperature was in the range 28.0–29.1°C (65,66).

Muscle : Type-I collagen is obtained from the muscle of fishes such as the Atlantic salmon,Amur sturgeon, catfish, carp, etc. (67). Atlantic salmon yielded 23.7% ASC, 70.5% PSC and 5.8% in-soluble collagen (ISC) (68). Amur sturgeon gave ASC 31.56%, PSC 58.49% and SSC(salt-solubilized collagen) 3.02% with a Td of 33°C (70). ASC 97.523 mg/g dwb and PSC 368.360 mg/g dwb was obtained from the Cultured Clarias species (hybrid of Clarias gariepinus×C. macrocephalus), a freshwater catfish (70). The other fishes used were eel, saury, mackerel , chum salmon, carp, etc. The Td of collagen from muscle was comparitively higher than that of skin (71).

Swim bladder : Collagen type-I obtained from the swim bladder of Marine cat fish (Tachysurus maculatus) yielded 35% of collagen in its lyophilised dry weight basis when PSC method

Table 1. Type I collagen obtained from the skin of various fishes.

Fish	Yield & type of collagen	Denaturation temperature	Ref
Japanese sea-bass	51.4%	25.0–26.5°C	(39)
Chub mackerel	49.8%	25-28°C	"
Bullhead shark	50.1%	"	"
Nile tilapia (Oreochromis niloticus)	38.84(N) and 20.70% (O) dwb(ASC) 48.21 (N) and 38.27%(O) dwb (PSC)	34.43 (O)and 4.29°C 3(N) (ASC) 34.61 (O) and 34.32°C (N) (PSC)	(40)
Malaysian catfish (hybrid *Clarias sp.gariepinus*)	ASC,PSC :18.11±0.32 and 26.69±0.54% (wwb)	31.5 and 31.0°C	(41)
Unicorn leatherjacket (Aluterus monoceros)	3.39 and 28.33%dwb (ASC & PSC)	30.03°C	(42)
Ocellate puffer fish	10.7% dwb (ASC) 44.7% dwb (PSC)	-	"
Grass carp	46.6% (PSC)	28.4°C	(43)
Blackcarp (Mylopharyngdon piceus)	45.7% dwb(PSC)	25.6°C	(44)
Nileperch (Lates niloticus)	Young fish: 63.1% Adult fish: 58.7% dwb(ASC)	36 °C	(45)
Leather jacket (Odonus niger)	46–50% (ASC) 49–58% (ASC and PSC) 64–71% (PSC)	Skin collagen - 27 to 28 °C bone collagen -31 to 32 °C	(46)
Brown stripe red snapper (Lutjanus vitta)	9%wwb(ASC) 4.7%wwb(PSC)		(47)
Blackpomfret (Parastromateusniger)	13.6% dwb(ASC)	-	(48)
Siganus sutor	12-14 %	-	(14)
Narroe barred	14-17%	-	"
Carcharhinus leucas	14-15%	-	"
Indopacificking mackerel	10-12%	-	"
Bigeye snapper (Priacanthus macracanthus)	6.4% & 1.1% wwb (ASC & PSC)	-	(49)
Rainbow trout (Onchorhynchus mykiss)	9.448% and 1.122% wwb (ASC)	-	(50)
Surf smelt (Hypomesus pretiosus japonicus brevoort)	24% dwb (ASC)	32.5°C	(51)
Barramundi (Lates calcarifer)	Pepsin (PSC) and papain (pASC) aided extraction 43.6% and 43.9%, ASC- 8.1% (dwb)	-	(52)
Double-spotted queenfish (Scomberoides lysan)	ASC, pdc 7.82, 3.92	-	(53)
Malabar grouper (Epinephelus malabaricus)	ASC, pdc 12.5 and 6.49%	-	"
Marine eel fish (Evenchelys macrura)	ASC-80%,PSC-7.1%dwb	38.5°C (ASC)35°C (PSC)	(32)

Where, ASC: Acid Soluble Collagen,PSC:Pepsin Soluble Collagen, N- Noitup method, O: Ogawa method, PDC: Pepsin Digestible Collagen, dwb:dry weight basis, wwb: wet weight basis.

Table 2. Type-I collagen extracted from the bones of different fishes.

Fish	Yield & type of collagen	Denaturation temperature	Reference
Skipjack tuna	42.3%	29.5–30.0°C	(46)
Japanese sea-bass	40.7%	-	"
Ayu	53.6%	-	"
Yellow sea bream	40.1%	"	"
Horse mackerel	43.5%	-	"
Black drum and sheepshead seabream	PSC	-	(54)
Rainbow trout (Onchorhynchus mykiss)	9.448% and 1.122%(ASC)	-	(50)

Table 3. Type-I collagen obtained from the scales of various fishes.

Fish	Yield & type of collagen	Denaturation temperature	Reference
Sardine	50.9% dwb (PSC)	-	(60)
Red sea bream	37.5% dwb (PSC)	-	"
Japanese sea bass	41%dwb (PSC)	-	"
Carp fish (Cyprinus carpio)	ASC, PSC	ASC: 32.9°c PSC :29.0°c	"
Grass carp(Ctenopharyngodon idellus)	25.64% dwb(PSC)	35-40°c	(61)
Spotted golden goatfish (Parupeneus heptacanthus)	0.46% & 1.2% dwb(ASC & PSC)	-	(62)

was used. Other fish swim bladder from which collagen is obtained includes Bester sturgeon fish,Rohu (Labeo rohita), Arius parkeri (Gurijuba), yellowfin tuna (Thunnus albacares),bighead carp (Hypophthalmichthys nobilis), Cynoscion acoupa (Pescada Amarela), Cynoscion leiarchus (Pescada Branca), Alaska pollack, etc. (72-76). Yellow fin tuna yielded 1.07% ASC and 12.10% PSC. The biological properties of the collagen obtained from the swim bladder of fishes are to be validated for their application as a polymeric material which is biocompatible in nature and has potential to be used for dressing wounds (in controlled drug delivery systems),as injectables in case of coating cardiovascular prostheses and as a support for cell growth.

Conclusion

Fish processing waste which is discarded after the step of processing poses a threat to the environment. It not only causes water pollution, but also air pollution due to the foul smell they emanate. FPW is presently used for various purposes such as production of gelatin, collagen, enzymes, etc. From the above review, we can conclude that fish waste (particularly its processing waste) is an excellent source of collagen especially type-I. And the use of this economic and abundant waste as a starting material for collagen production would also contribute for the protection of our environment. The collagen type I has already been used in various fields such as medical (as dressing material for wound healing), pharmaceutical (as drug delivery systems), biomedical (as biomaterial for tissue engineering purposes), etc. to name a few. Many more such applications of type-I collagen are yet to be identified and utilized for the welfare of mankind. Apart from the type-I, other types of collagen have also been obtained from their wastes. Extensive studies have been carried out till date for extracting collagen from fishes and other marine source as well (including vertebrates and invertebrates). If the research on collagen isolation is intensified further, it may reveal even better and more reliable sources of collagen type I with promising yields.

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