World Journal of Fish and Marine Sciences 2 (5): 471-474, 2010
ISSN 2078-4589

Isolation and Characteristics of Collagen from Fish Waste Material

Ali Aberoumand

Department of Fisheries, Natural Resources College, Behbahan High Educational Complex, Iran

Abstract: Collagen is the most abundant protein of animal origin, comprising approximately 30% of total animal protein. There are at least 19 variants of collagen, named type I to XIX. Types I, II, III and V are the fibrous collagens. Type I collagen is found in all connective tissue, including bones and skins. It is a heteropolymer of two "1 chains and one "2 chain. It consists of one-third glycine, contains no tryptophan or cysteine and is very low in tyrosine and histidine. Guanidine hydrochloride to solubilize the part of collagen referred as GSC and then RS-AL (crude connective tissue fractions) digested with pepsin called PSC were extracted from the fish tissues. Applications of fishes collagen is different in different industries.

Key words: Collagen % Fish skin % Extraction produces % Collagen properties

INTRODUCTION

Collagen is an important biomaterial in medical applications due to its special characteristics, such as biodegradability and weak antigenecity. Thus collagen, as a new type of biomaterial, has been used in drug delivery systems 2 and tissue engineering. In addition, there are some intrinsic relationships between collagen and many diseases such as rheumatoid arthritis4 and systemic sclerosis [1-5]. Gelatin can be made into roll film and drug capsules and can also be used as a biomaterial in biomedical applications, such as in drug delivery systems.6 which are very different from the traditional capsule. C ollagen hydrolysate is a polypeptide composite made by further hydrolysis of denatured collagen. It has been used in cosmetics and as a food additive [6-9]. Many attempts have been made to utilize the limed split wastes in ways other than utilization as the source of commercial gelatins. It had been shown that the collagen extracted from calf limed splits at a low temperature by the pepsin-digestion method had properties similar to those of the commercial collagen [10, 11]. In addition, collagen polypeptide had also been prepared from the limed hide offal in a two-step protease (EA and EA 537) treatment process at 45-60°C [12].

Collagen is the most abundant protein in vertebrates making up approximately 30% of total protein. Collagen is a major component of connective tissue, muscle, teeth, bone and skin. There are 19 types of collagen, labeled I-XIX. Collagen is composed of three similarly sized triple helix polypeptide chains. Each chain contains about 1000 amino acid residues in size and has an average length of 300 nm and a diameter of 1.4 nm. Collagen has a repetitive primary sequence of which every third residue is glycine. The sequence of the polypeptide chain can be described as Gly-X-Y, in which X and Y are often found to be proline and hydroxyproline forming a left-hand super helix with the other two chains [13]. Collagen has been used in the biomedical, pharmaceutical [14], food and cosmetic industries [15]. Most commercial collagens come from bovine skin, pig skin or chicken waste. These land animal sources are unsuitable for many religious and ethnic groups, face regulatory and quality control difficulties and can contain biological contaminants and poisons, such as BSE (Mad Cow Disease), transmissible spongiform encephalopathy (TSE) and foot-and-mouth disease (FMD). Thus, it is of interest to search for new sources of collagen originating from fish and other seafood [16].

The three major methods of collagen extraction produce neutral salt-solubilized collagen, acid-solubilized collagen and pepsin-solubilized collagen [17]. Many researchers have studied the pepsin-solubilized collagen (PSC) extraction method from different sources, such as from the skin of brownstripe red snapper [18], fish waste material [19], albacore tuna and silver-line grunt skin, bone and scale of black drum and sheepshead seabream and black drum and sheepshead seabream skin [20]. Some of the methods are performed at 4°C using a high-speed centrifuge. However, some are performed at a

Corresponding Author: Ali Aberoumand, No: 22, Second Alley of Ab Va Barg, Zolfegari Street, Behbahan , Kuzestan, Iran
E-mail: Aberoumand38@yahoo.com.

higher temperature and use lower speed centrifugation. Noitup *et al.* [21] extracted a high yield of collagen (90%) from silver-line grunt and albacore tuna. This method (hereafter referred to as the Noitup method) was performed at 4-6°C for the whole process with a centrifuge speed of 30 000 g, while Ogawa *et al*. [22] used a method (hereafter referred to as the Ogawa method) involving a higher temperature of 22-23°C, with a centrifugation speed of 10,000 g. Thus, the Ogawa method seemed to be more convenient for a pilot scale. However, the biochemical properties and thermal stability of collagen extracted by these two methods might be different. Therefore, the objective of this study was to compare the biochemical and physical properties of collagen extracted by these two methods in order to select the appropriate method for extraction of collagen from Nile tilapia skin to be used as a moisturizing cream ingredient.

DISCUSSION

The amino acid contents of the fish skin were less than those of bovine collagen (206.60 g /1000g), which was correlated to the lower denaturation temperature of fish collagen compared to bovine collagen. Li *et al*. [23] reported that collagen from land animals had a higher denaturation temperature than that from fish because of its higher amino acid content. The ASC and PSC from Nile tilapia had a higher denaturation temperature compared with the other fish varieties reported [24]. In addition, for aquatic animals, habitat and body temperature affected the denaturation temperature of collagen [25]. Jongjareonrak [19] reported that three factors affected the collagen functional properties: aging and the living period of fish samples; the processing steps for raw fish; and the pH and NaCl concentrations during the collagen extraction step. In the current study, both methods used Nile tilapia fish skin and had controlled pH and NaCl concentrations. Kolodziejska *et al*. [28] reported that temperature correlated with the collagen extraction yield. The solubilization of collagen increased according to increasing temperature and completely solubilized at 45°C. Moreover, the extraction temperature of the Ogawa and Noitup methods was 22-23 and 4°C, respectively, which was lower than the denaturation temperature.

Methods used for isolation of collagen from cattle skins are not effective enough for the isolation of collagen from fish, cuttlefish, squid skins etc. There are only a few works dealing with the practical utilization of connective tissue offal of marine vertebrates and invertebrates [26] Other works, concerning collagen of marine animals focus mainly on cognitive aspects, such as collagen content in tissues, its genetic types, amino-acid composition, extent of intra and intermolecular cross linking, susceptibility to endo and exogenous enzymes and thermal stability of collagen Nagai and Suzuki [20]. Isolated about 2% of ASC and 35% of PSC from the skin of S. lycidas on dry weight basis. From the skin of Japanese Sea bass, Chub mackerel and Bullhead shark, the yield of collagens was very high and the values were about 51.4%, 49.8% and 50.1% respectively on the basis of lyophilized dry weight [27, 28].

Tonar and Marko [32] studied the yield of 51±3.4/28±1.8 and 50±3.3/36±2.4 of collagen connective tissue. The collagen content may be decreased due to denaturation of protein during the process of methodology and difference in environmental temperature [29].

The collagen content in the skin of Baltic cod (Gadus morhua) was 21.5% on the wet weight basis and about 71.2% on the dry weight basis [30] both the collagen and non-collagen protein content in cod skins depends upon the fishing season. During starvation albumins and globulins are degraded and the collagen content in skins increases [31] extracted 60% of PSC and only 12% of ASC from the cartilage of S. officinalis on wet weight basis whereas, in the same animal. In O. vulgaris, extracted 1.4% and 1.9% of collagen from the arm and mantle muscles and the protein content was also reported as 9.1% and 14.0% respectively. But in the present study 0.48% of GSC and 1.28% of PSC (Wet weight basis) was extracted from the N. crepidularia. Sadowska *et al*. [32] studied and established that the gross composition of skin of cod caught in the same season in different years is almost constant and the collagen content in the skin amounts an average to 21.5% in the wet weight and 71.2% in the dry weight. It was also absorbed that the share of collagen in total protein was considerably determined on the basis of hydroxyproline in samples. Further in the skin of cod the non collagen proteins, peptides and amino acids were estimated as 4.9% and 16.3% respectively on a wet and dry weight basis. Some studies on collagen reveals that the collagen represents the chief structural protein accounting for approximately 30% of all vertebrate body protein. The major impediment in the dissociation of collagen type I from tissue is the presence of covalent crosslinks between molecules. Collagen is insoluble in organic solvents [33]. In some tissues, notably in skins of young animals, crosslinking is sufficiently low to extract a few percent under appropriate conditions. The most commonly used solvents are dilute acetic acid or neutral salt solution (0.8M NaCl). The acetic acid is used to extract fresh and negligible cross linked collagen molecules present in the outer skin of the animal. The extracted material is purified by precipitation, centrifugation and dialysis [33]. In the present study also, acetic acid was used for the extraction. In conclusion, this review paper showed that collagen content in the skins of fishes was high depends upon the fishing season. Collagen has been used in the biomedical, pharmaceutical, food and cosmetic industries and also it can convert to gelatin. Gelatin is high valuable compound. Fishes collagen was better that other animal's collagen from point of view of qualitative and quantitative.

REFERENCES

1. Lee, C.H., A. Singla and Y. Lee, 2001. Biomedical applications of collagen. Int. J. Pharm., 221: 1-22.
2. Friess, W., 1998. Collagen - biomaterial for drug delivery. Eur. J. Pharm. Biopharm., 45: 113-136.
3. Pachence, J.M., 1996. Collagen-Based Devices for Soft Tissue Repair. J. Biomed. Res. (Applied Biomaterials), 33: 35-40.
4. Holmdahl, R., R. Bockermann and J. Backlund, 2002. The molecular pathogenesis of collagen-induced arthritis in mice - a model for rheumatoid arthritis. Ageing Res. Rev., 1: 135-147.
5. Tamby, M.C., Y. Chanseaud and L. Guillevin, 2003. New insights into the pathogenesis of systemic sclerosis. Autoimmunity Rev., 2: 152-157.
6. Einersona, N.J., K.R. Stevensa and W.J. Kao, 2002. Synthesis and physicochemical analysis of gelatin-based hydrogels for drug carrier matrices. Biomaterials, 24: 509-523.
7. Langmaier, F., M. Mladek and K. Kolomaznik, 2002. Isolation of elastin and collagen polypeptides from long cattle tendons as raw material for the cosmetic industry. Int. J. Cosmet. Sci., 24: 273-279.
8. Langmaier, F., M. Mladek and K. Kolomaznik, 2001. Collagenous hydrolysates sources of proteins. Int. J. Cosmet. Sci., 23: 193-199.
9. Morimura, S., H. Nagata and Y. Uemura, 2002. Development of an effective process for utilization of collagen from livestock and fish waste. Proc. Biochem., 37: 1403-1412.
10. Li, G.Y., S. Fukunaga and K. Takenouchi, 2003. Physicochemical properties of collagen isolated from calf limed splits. J. Amer. Leather Chem. Ass., 98: 224-229.
11. Li, G.Y., S. Fukunaga and K. Takenouchi, 2003. Physioligical and cell biological properties in vitro of collagen isolated from calf limed splits. J. Soc. Leather Technol. Chem., 88: 66-71.
12. Li, Y.C., D.Y. Zhu and L.Q. Jin, 2005. Preparation and analysis of collagen polypeptide from hides by enzymes. J. Soc. Leather Technol. Chem., 89: 103-106.
13. Whitford, D., 2005. Protein Structure and Function. John Wiley & Sons Ltd., England, pp: 528.
14. Lee, C.H., A. Singla and Y. Lee, 2001. Biomedical applications of collagen. Inter. J. Pharma., 221: 1-22.
15. Senaratne, L.S., P.J. Park and S.K. Kim, 2006. Isolation and characterization of collagen from brown backed toadfish (Lagocephalus gloveri) skin. Bioresource Tech., 97: 191-197.
16. Kim, S.K. and E. Mendis, 2006. Bioactive compounds from marine processing byproducts - a review. Food Research Intl., 39: 383-393.
17. Ward, A.G. and A. Courts, 1977. The Science and Technology of Gelatin. Academic Press, London, pp: 564.
18. Zhang, Y., W. Liu, G. Li, B. Shi, Y. Miao and X. Wu, 2007. Isolation and partial characterization of pepsin-soluble collagen from the skin of grass carp (Ctenopharyngodon idella). Food Chem., 103: 906-912.
19. Jongjareonrak, A., 2006. Characterization and Functional Properties of Collagen and Gelatin from Bigeye Snapper (Priacanthus macracanthus) and Brownstripe Red Snapper (Lutjanus vitta) Skins. Ph.D. Thesis. Prince of Songkla University, pp: 155.
20. Nagai, T. and N. Suzuki, 2000. Isolation of collagen from fish waste material - skin, bone and fins. Food Chem., 68: 277-281.
21. Noitup, P., W. Garnjanagoonchorn and M.T. Morrissey, 2005. Fish skin type I Collagen characteristic comparison of albacore tuna (Thunnus alalunga) and silver-line grunt (Pomadasys kaakan). J. Aquat. Food Prod. Technol., 14(1): 17-27.
22. Ogawa, M., M.W. Moody, R.J. Portier, J. Bell, M.A. Schexnayder and J.N. Losso, 2003. Biochemical properties of black drum and sheepshead seabream skin collagen. J. Agri. Food Chem., 51: 8088-8092.
23. Li, H., B.L. Liu, L.Z. Gao and H.L. Chen, 2004. Studies on bullfrog skin collagen. Food Chem., 84: 65-69.
24. Ciarlo, A.S., M.E. Paredi and A.N. Fraga, 1997. Isolation of soluble collagen from hake skin (Merluccius hubbsi). J. Aquat. Food Prod. Technol., 6(1): 65-77.

25. Nagai, T., Y. Araki and N. Suzuki, 2002. Collagen of the skin of ocellate puffer fish (Takifugu rubripes). Food Chem., 78: 173-177.
26. Sadowska, M., I. Kolodziejaska and C. Niecikowska, 2003. Isolation of collagen from the skin of Baltic cod (Gadus morhua). Food Chem., 81: 257-262.
27. Montero, P., C. Alvarez, M.A. Marti and J. Borderias, 1995. Plaice skin collagen extraction and functional properties. J. Food Sci., 60(1): 1-3.
28. Kolodziejska, I., Z.E. Sikorski and C. Niecikowska, 2008. Parameters affecting the isolation of collagen from squid (Illex argentinus) skins. Food Chem., 66: 153-157.
29. Nagai, T. and N. Suzuki, 2000. Isolation of collagen from fish waste materials - skin, bone and fins. Food Chem., 68(3): 277-281.
30. Sadowska, M., I. Kolodziejska and C. Niecikowska, punctaus). Food Chem., 101: 621-625. 2003. Isolation of collagen from the skins of Baltic cod (Gadus morhua). Food Chem., 81: 257-262.
31. Sikorski, Z.E. and D.N. Scott, 1984. Buisson DH. The role of collagen in quality and processing of fish. Critical Rev. Food Sci. Nutri., 20(4): 301-343.
32. Tonar, Z. and A. Marko, 2004. Microscopy and Morphometry of Integument of the Foot of Pulmonate Gastropods Arion rufus and Helix pomatia. Acta Vet. Brno., 73: 3-8.
33. Amit Kumar, C., 2008. Isolation and partial characterization of collagen from the whole body tissues of Perna viridis Linnaeus, 1758. M.Sc. Thesis, Annamalai University, Parangipettai, pp: 1-44.