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# Extraction and characterization of acid- and pepsin-soluble collagen from scales of marine and freshwater fishes caught from temperate and sub-tropical countries

**Sheik Md. Moniruzzaman, Kigen Takahashi, Emiko Okazaki and Kazufumi Osako ***

*Department of Food Science and Technology, Tokyo University of Marine Science and Technology, 4-5-7 Konan, Minato-ku, Tokyo 108-8477, Japan*
* Correspondence: osako@kaiyodai.ac.jp; Tel.: +81-03-5463-0620

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## Introduction

Food, pharmaceutical and cosmetic industries around the world are observing a growing demand for collagen. The widely-used mammalian collagen (porcine and bovine) has significant limitations due to socio-cultural, religious and health-related concerns. Moreover, intense competition exists between manufacturers for acquisition of mammalian sources, which led to increased demand and excessive costs. These urged scientists to find and develop alternatives to mammalian collagen. The fish collagen is not associated with BSE and is acceptable for Islam. Furthermore, the sources are discarded wastes (i.e., scales, skin etc.) of fish processing industries. Therefore, production and application of fish collagen pleases the needs of human consumption as well as reduces pollution.

It is assumed that habitat temperature affects the properties of collagen and collagen from warm water fish has higher thermal stability. However, no information exists for acid- and pepsin-soluble collagen (ASC and PSC) from scales of temperate and sub-tropical country fishes. Considering the above-mentioned phenomena, the aim was to clarify the effect of environmental temperature on collagen properties from scales of marine lizardfish, *Saurida* sp. (Japan and Vietnam) and freshwater carp, *Cyprinus carpio* (Japan and Bangladesh) fishes.

## Materials and methods

After descaling of collected fish samples, scales were washed with cold distilled water, transported to Food Processing Laboratory, TUMSAT and immediately stored at -30ºC. Prior to analysis, scales were cut into small pieces if necessary. Moisture, ash and crude protein content was measured following the method of AOAC [1]. For crude protein content calculation, conversion factor 5.95 was used [2].

Extraction of ASC and PSC was determined according to Nagai and Suzuki [3] with slight modifications. After pre-treatment (removal of non-collagenous proteins and demineralization), ASC was extracted with 0.5 M acetic acid with the ratio of 1:3 (w/v) for 3 days. Then the extract was centrifuged at 20000 × g for 1 h and obtained supernatant was salted out by NaCl to a final concentration of 2.6M in presence of 0.05M Tris-HCl (pH 7.5). Resulting precipitate was collected by centrifugation and dissolved by minimum volume of 0.5 M acetic acid and dialyzed against 0.1M acetic acid and distilled water for 48 h respectively. Resulting dialysate was freeze-dried and referred to as ASC. For undissolved residue after acid extraction, 1% pepsin was used at the sample/solvent ratio of 1:10 (w/v) for 48 h. All processes were performed at 4ºC. The yield of collagen was determined by hydroxyproline content in raw fish scales and extracted collagen.

Ten-twenty mg of collagen was hydrolyzed in 6M HCl at 110ºC for 22 h under vacuum and amino acid composition was analyzed by HPLC. The SDS-PAGE of ASC and PSC were determined by Laemmli [4] with slight modifications. Both collagens were loaded onto polyacrylamide gel (7.5%) and high molecular weight markers was used to estimate the molecular weight of proteins. Denaturation temperature was analyzed using differential scanning calorimetry (DSC) according to Kittiphattanabawon et al. [5].

## Results and discussion

The moisture content was above 50% in all samples. The crude protein content of carp scales was higher than that of lizardfish scales. In contrast, higher ash contents were found in lizardfish scales than carp scales

(Moniruzzaman, unpubl. data). Immersing the sample in 0.1M NaOH for 6 hours removed the non-collagenous proteins and treatment with 0.5M $Na_2EDTA$ solution at pH 7.5 for 24 hours effectively removed ash content from scales.

The yield of pepsin soluble collagen was higher compared to acid soluble collagen. It was suggested that pepsin facilitated the extraction of collagen via the cleavage of telopeptide region [6].

Glycine was the dominant amino acid found in ASCs and PSCs from sampled fish scales and both collagen contained major amino acids which coincides with collagen of other fish species [2,7,8]. However, no tryptophan content could be detected in all collagen samples.

The SDS–PAGE pattern revealed that both ASCs and PSCs were type I collagens (consisted of two different α chains, α1 and α2 and density of α1 is higher than α2). High molecular weight component, β chain was also observed. Collagen isolated from scales of other fish species like deep-sea redfish [2], spotted golden goatfish [7], Croceine Croaker [9], horse mackerel, grey mullet, yellowback seabream [8] and seabass [10] have also been categorized as type I collagen.

## Conclusions

ASC and PSC could be extracted from the lizardfish and carp scales caught in Japan, Bangladesh and Vietnam. Comparison between the properties of collagen from fish scales between temperate and sub-tropical countries was denoted and results from this study imply that collagen from these fish scales might be an alternative source of mammalian derived collagen.

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