Soluble collagen from fish (sardine) scales was yielded at about 5% with 0.5 M acetic acid after demineralization with EDTA, while a great portion of the collagen remained insoluble. The solubility of this insoluble collagen was about 20% at 45°C (denaturation temperature of soluble collagen) for 24 h. The remaining 80% of the insoluble collagen was denatured in the form of insoluble gelatin, and that may be an interesting food material.

Key words: collagen; scale; denaturation temperature; DSC; optical rotation

Type I collagen is the main component of extracellular matrix and has common functions such as physical protection of tissues and organs or physiological regulation of the cell environment for many species of animals. In spite of that, some species-specific characteristics should be taken in regard. The industrial use of type I collagen has expanded over a diverse field, in foods, cosmetics, and biomedical materials. Although the main sources of industrial collagen are now limited to those from bovine or pig skin type I collagen, the collagen source should also be expanded to other than land vertebrates, for example those from aquatic animals, because collagen from aquatic animals have some important features for food processing that are distinguished from land vertebrate collagens. The greatest features of fish collagen are a lower denaturation temperature and viscosity than land vertebrate collagens. There is little study of fish scales, however, except that Kimura showed the presence of type I collagen in fish scales. A great amount of fish scales is produced in sardine fish processing factories in Japan and has potential as an important collagen source. In this paper, we show the native collagen can be extracted and purified from the fish scales with relatively simple procedures and we would like to propose the use of scale collagen as a food material.

Fish scales were collected in a fresh sardine processing factory. The collagen extraction was done after demineralization, by the method by Araya. The degree of demineralization with different solvents and the number of extractions was investigated. Two different solvents were tested: (1) 0.05 M Tris–HCl buffer (pH 7.5) containing 0.5 M EDTA and (2) 0.2 N HCl. The dissolved calcium and protein were measured by colorimetry and the microbiuret method, respectively. The thoroughly demineralized material was extracted with 0.5 M acetic acid to obtain soluble collagen (SC). The recovery of collagen from the solution was done by adjusting to pH 7. The final residue (insoluble collagen, IC) was treated with 1 M phosphate buffer at pH 7 and washed with distilled water. All the isolation procedures were done at 4°C.

To raise the yield of soluble collagen from IC, pepsin digestion was done, changing the time and temperature of the enzyme reaction: IC was suspended in 0.5 M acetic acid, and was pepsin added (pepsin/IC = 1/100, w/w; pepsin, 3200 units/mg solid, Sigma, U.S.A.). The reaction temperature was 5–20°C and the incubation was continued for 72–96 h. The recovery and purification of pepsin-solubilized collagen (PSC) from the solution was done following the preparation methods for soluble collagen. The yield of PSC was measured by the protein contents of the supernatant by microbiuret method. There is a fear that low molecular weight components are produced by solubilizing of collagen from aquatic animals with pepsin. PSC from different conditions were characterized by SDS-PAGE.

As in our previous paper, the denaturation temperature of SC in 0.5 M acetic acid was measured on a polarimeter (SEPA-200, digital type, Horiba, Tokyo) at 589 nm. Differential scanning calorimetry (DSC) of SC was done using a DSC apparatus (SSC-560U, Seiko, Tokyo) coupled with a thermal analysis data system 560DSIII as described previously. The sample for DSC was prepared by adjusting the SC solution in 0.5 M acetic acid to neutral pH with 0.5 M sodium hydroxide. Pig skin collagen that was prepared as according to our previous paper was compared to scale collagen.

To test the solubility of solid collagen in m/15 phosphate buffer (pH 7), solid collagen was incubated at a specified temperature from 5 to 45°C for 24 h. The suspension was centrifuged at 18,000 rpm (36,000 × g) for 1 h, and the protein contents of supernatant were measured by the microbiuret method.

To prepare the soluble collagen, the demineralization of scales was an important process. The extraction of calcium and protein, with two different solvents (0.5 M EDTA and 0.2 N HCl) were investigated. At the first extraction, the extraction of calcium with both solvents came up to about 90%. However the extraction of protein with 0.2 N HCl was higher than that with 0.5 M EDTA. In case of the demineralization by 0.2 N HCl, scale collagen may have undergone a partial hydrolytic deterioration. To prepare the SC and IC, the demineralization was done with neutral solvent (0.5 M EDTA). Thus by EDTA extraction thrice, about 97% of the calcium were removed from scales. After removal of calcium, SC was extracted with 0.5 M acetic acid at about 5% yield to total collagen contents. The relatively low yield of SC may be considered as unsatisfactory for a food material. However the SC is important as a reference for characterization of the properties of the main part of scale collagen. The purification of SC was done by changing the pH of the collagen solution. The advantage of

<table>
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<th>Temp. (°C)</th>
<th>Time (h)</th>
<th>Yield (%)</th>
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<tr>
<td>20</td>
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<td>83.5</td>
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<td>96</td>
<td>29.2</td>
</tr>
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<td>5</td>
<td>96</td>
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PSC, pepsin solubilized collagen; IC, insoluble collagen. * PSC/IC.

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this method is the ease of removal of the salt content of SC in the subsequent step.

Ninety five % of fish scale collagen remained insoluble in the EDTA demineralization process. To raise the extraction of collagen from the demineralized material, the conditions for pepsin digestion were investigated (Table). The enzyme reaction at above 25°C produced solubilized collagen (pepsin solubilized collagen, PSC) containing a considerable amount of low molecular weight peptides, indicating a partial denaturation of collagen occurred during pepsin digestion. SDS-PAGE patterns of PSC showed that lower molecular weight fragments than z1(I) chain were the main components, under pepsin-digestion conditions at 20°C above 72 h and at 15°C above 96 h (data not shown). The enzyme reaction below 10°C gave only an unsatisfactory yield of PSC (below 30%) within the reaction time given for this experiment. The optimum conditions of pepsin digestion were at 15-20°C for 72 h (70-80% yield), although the longer reaction time produced a certain amount of low molecular weight components. This high yield of PSC should be taken as a feature of scale collagen compared with that of land animals, although the time and temperature of enzyme reaction was carefully considered. Pepsin treatment is the usual method for the preparation of soluble collagen, and reduces its antigenicity. However, the self-assembly of PSC was weaker than that of SC, and the most im-

![Fig. 1. Denaturation Curves of Scale and Pig Skin Type I Collagen as Measured by Specific Rotation.](image1)

![Fig. 2. DSC Curves of Scale and Pig Skin Type I Collagen.](image2)

portant problem was the production of lower molecular weight fragments than z1(I) chain from collagen. Scale collagen was easier to make into the low molecular weight fragments than that of land vertebrates.

Kimura et al. estimated21 and we also confirmed that the obtained scale collagen had the characteristics of type I collagen by SDS-PAGE and amino acid compositions. However, scale collagen also had the unique feature of a lower content of imino acid than that from land mammalian type I collagen.21

The optical rotation change of SC in the solution started at 23°C, and finished at 30°C. Thus the denaturation temperature (mid-point, Tm) of the SC solution was estimated as 27.3°C (Fig. 1) and was lower by about 13°C than pig collagen. Another feature of SC denaturation is that the whole change of optical rotation occurred in a narrower range of temperature. Such a narrow width of rotation change reflects the stronger cooperative nature of the SC molecular structure. This may indicate an interesting feature in term of food material properties since SC can transform more rapidly from the native state to gelatin form. The denaturation of shark collagen in the solution started at about 25°C, and finished at 32°C.21 The Tm of shark collagen was also equal to that of SC. It is considered that the denaturation behavior of SC is common to that of aquatic animal collagen.

DSC curve of SC indicated one peak, and the denaturation temperature was estimated as about 44°C (Fig. 2). The low denaturation temperature of SC is interesting for food applications. The denaturation temperature of SC was lower about 10°C than that of pig collagen gel. A few papers has reported that the denaturation temperature of collagen from aquatic animals is around 40°C.11 So the thermal stability of SC is not different from other aquatic animal type I collagens.

The solubility of IC and SC powder was measured by incubating it in the phosphate buffer at pH 7.0 at a specified temperature (Fig. 3). The melting curve of IC was different from that of SC. The dissolution of SC started at about 20°C. Above 30°C, the solubility of SC rose rapidly, and most of the SC was dissolved. In contrast, the solubility of IC was merely about 20% at 45°C. As judged by DSC of SC (Fig. 2), IC must be denatured substantially at 45°C, hence the remained 80% part of IC (Fig. 3) also must be denatured in the form of insoluble gelatin like a shark fin. This type of gelatin may also provide an interesting food material, such as dietary fiber.

References


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