Available online at www.sciencedirect.com
SCIENCE DIRECT
Food Chemistry 100 (2007) 477–481
Food Chemistry
www.elsevier.com/locate/foodchem

Behaviour of connective tissue in fish surimi on fractionation by sieving

Shoshi Mizuta *, Kojiro Nakashima, Reiji Yoshinaka

Department of Marine Bioscience, Faculty of Biotechnology, Fukui Prefectural University, Obama, Fukui 917-0003, Japan

Received 27 June 2005; received in revised form 20 September 2005; accepted 20 September 2005

Abstract

The surimis prepared from the three fish species, red barracuda *Sphyraena pinguis*, yellow sea bream *Dentex tumifrons*, and spotted shark *Mustelus manazo*, were fractionated by sieving through a 30-mesh stainless steel sieve. Chemical analyses revealed significant differences in the collagen content among the residual fraction on the sieve (fraction A), the passed fraction (fraction C), and the original surimi before sieving (fraction B): the fractions A and B showed values 10–20 and 5–10 times higher than those of the fraction C, respectively, for all the species examined. Histological observation indicated the richness of the thick connective tissues derived from myocommata in fractions A and B, while thick connective tissue was hardly observed in fraction C. These results suggested that the present fractionation method may have an effect of reducing the collagen content to about 10–20% of the original value, and that a large part of collagen in fish surimi exists in the relatively thick connective tissue, probably derived from myocommata.

Keywords: Collagen; Connective tissue; Surimi; Fish; Muscle; Myocommata

1. Introduction

Connective tissues in fish meat have been histologically classified as myocommata, perimysium and endomysium (Bremner & Hallett, 1985). Myocommata are thick collagenous sheets which separate fish muscle into myomeres. Perimysium and endomysium are relatively thin fibers which surround the muscle fiber bundle and individual muscle fiber, respectively. In Japan, many large-scale manufacturers of surimi or surimi-based products have made efforts to remove the connective tissues from fish meat by mincing or sieving, mainly because it has undesirable effects (unhomogeneity or granularity) on the texture of the final products, and collagen, which is a major proteinaceous component of connective tissue, is not an essential component for the formation of the gel structure. On the other hand, the removed fraction, which is very rich in connective tissue, is partially utilized as the material of surimi-based products with unique texture. The residual matter after recovery of surimi from shark meat by a meat separator is called "Suji" in Tokyo, and the products made from the "Suji" are also called "Suji", being one of the special products of the Tokyo area in Japan. In this context, the fractionation process appears to be very important for the removal or concentration of connective tissues in the manufacture of surimi, although there are small-scale manufacturers who adopt a manufacturing process without the removal of connective tissue. Few studies have, however, centered on the characteristics of collagen in the consequent fractions after the fractionating operations. In the present study, we tried to construct a model experimental system of fractionation of connective tissue in fish surimi and to characterize partially the connective tissue and collagen in the consequent fractions.

2. Materials and methods

2.1. Preparation and fractionation of surimi

Three species of fish, listed in Table 1, were obtained fresh within 24 h after death from a local market. The outline of the procedures used is shown in Fig. 1. All operations were carried out in a cold room at 4 °C. The flesh

* Corresponding author. Tel.: +81 770 52 6300; fax: +81 770 52 6003.
E-mail address: mizuta@fpu.ac.jp (S. Mizuta).

0308-8146/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2005.09.068

Table 1
Data of samples

Species	Month of sampling	Number of samples	Average body length (mm)	Average body weight (g)
Red barracuda (*Sphyraena pinguis*)	October	20	297	224
Yellow sea bream (*Dentex tumifrons*)	November	20	161	153
Spotted shark (*Mustelus manazo*)	November	1	750	1200

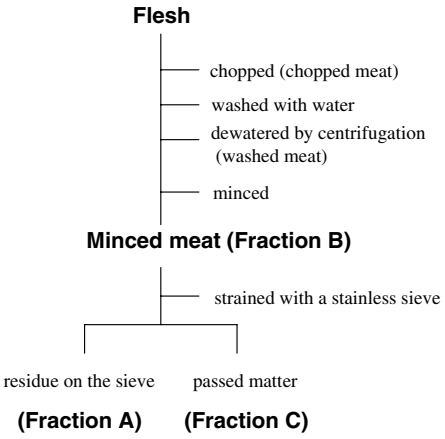

Fig. 1. Outline of the preparation procedure of surimi from the three fish species.

was dissected out, chopped with a knife (chopped meat), washed with water in a polyethylene bucket, dewatered by centrifugation (Model H-122; Kokusan Corporation, Tokyo, Japan) at 1500 g for 40 min (washed meat), and then minced by a hand mincer (Model C-1530; Pearl Metal Co. Ltd., Niigata, Japan). The minced meat (about 200 g), which was used as the surimi preparation in the present study, was sieved through a 30-mesh stainless steel sieve (Tokyo Screen Co. Ltd., Tokyo, Japan) with a plastic paddle. The passed fraction (about 100 g) was referred to as fraction C, and the fraction remaining on the sieve (about 100 g) as fraction A. The original minced meat prior to sieving was referred to as fraction B.

2.2. Preparation and determination of collagens

The chopped meats and fractions A–C (each 20 g) were homogenized in 5 volumes (v/w) of 0.1 M NaOH, and extracted in the same solution for 24 h with gentle stirring at 5 °C. The extraction was done to remove non-collagenous proteins and to prevent the effects of endogenous proteases on collagen according to the previous report (Yoshinaka et al., 1985). The residue after the alkali extraction (RS-AL) was thoroughly washed with distilled water, extensively homogenized in distilled water by a microhomogenizer (Polytron; Kinematica, Luzern, Switzerland), and then lyophilized. The lyophilized samples were hydrolyzed in 6 M HCl at 130 °C for 3.5 h. The hydroxyproline content in the hydrolysate was determined by the method of Woessner (1961). The collagen content in the samples prepared from the yellow sea bream meat was determined by multiplying the hydroxyproline content by a converting factor, 8.6, which had been precalculated from the amino acid composition of the Type I collagen from yellow sea bream muscle (Yata, Yoshida, Fujisawa, Mizuta, & Yoshinaka, 2001). The hydroxyproline content in fish collagens is known to vary among fish species (Sato, Yoshinaka, & Sato, 1989), but the collagen content of the samples prepared from the red barracuda and spotted shark meats was also estimated by the same way using the factor, 8.6, in the present study.

The RS-ALs, prepared as above, were also analyzed by SDS-polyacrylamide gel electrophoresis (PAGE) as described later.

2.3. Chemical analysis

Protein nitrogen in 5% trichloroacetic acid-insoluble fraction was determined by the micro-Kjeldahl method. The value of 6.25 was used as the factor for converting the nitrogen content to protein content. Moisture was determined by drying the sample to a constant weight in an oven at 110 °C.

2.4. SDS-PAGE

SDS-PAGE was performed by the method of Laemmli (1970) using 7.5% polyacrylamide gels. The samples (about 5 μg) were applied to sample wells and electrophoresed, along with a mixture of standard proteins (SDS-6H, Sigma Chemical Corporation, St. Louis, MO, USA). Gels were stained for protein with Coomassie Brilliant Blue (CBB) R-250 essentially as described by Fairbanks, Steck, and Wallach (1971). The gel was initially stained by soaking in 10% acetic acid containing 0.05% CBB R-250 and 25% 2-propanol for 1 h at room temperature. The first staining solution was then exchanged for 10% acetic acid containing 0.004% CBB R-250 and 10% 2-propanol. After 2 h, the gel was soaked in 10% acetic acid containing 0.002% CBB R-250 for 2 h. Then the background of the gel was extensively destained with 10% acetic acid. The collagen and related peptides were stained metachromatically and non-collagenous proteins were stained orthochromatically (Duhamel, 1983; Micko & Schlaepfer, 1978).

2.5. Histological observations

Histological observation with a light microscope was carried out essentially as described previously (Mizuta, Yoshinaka, Sato, & Sakaguchi, 1994). The sample was fixed in Bouin's solution (Kiernan, 1990) for 6 h at room

temperature and embedded in paraffin (Parahisto, Nacalai Tesque, Kyoto, Japan). Sections (thickness: 4 μm) were cut with a microtome. The prepared sections were stained with Azan stain (Kiernan, 1990) and examined with a light microscope (Optiphoto-2, Nikon, Tokyo, Japan).

3. Results and discussion

Table 2 shows the water, protein, and collagen content of the material meats and fractions A–C presently prepared for the three fish species. The flesh from red barracuda, yellow sea bream, and spotted shark showed collagen content of 0.49%, 0.42%, and 0.73% of wet sample, 1.78%, 2.07%, and 3.19% of dry sample, and 3.05%, 2.65%, and 4.52% of protein, respectively. The fraction A from them showed collagen contents of 0.89%, 0.80%, and 2.8% of wet sample, 4.08%, 4.72%, and 13.8% of dry sample, and 6.05%, 5.46%, and 14.0% of protein, respectively, which were approximately 2–2.5 times higher than those of the fraction B (0.35%, 0.37%, and 1.24% of wet sample, 1.63%, 2.33%, and 7.08% of dry sample, and 2.52%, 2.61%, and 7.42% of protein). While the collagen contents of the fraction C (0.07%, 0.07%, and 0.11% of wet sample, 0.30%, 0.41%, and 0.66% of dry sample, and 0.46%, 0.46%, and 0.71% of protein) were about 1/5–1/10 of those of the fraction B. These results suggest that the present method of fractionation may have the effect of reducing the collagen content to about 10–20% of the value of the original surimi (fraction B). Incidentally, collagen was effectively concentrated in the fraction A showing collagen content of more than twice that of the original value.

Fig. 2 shows the SDS-PAGE analyses of the fractions A–C along with those of the RS-AL and fleshes. The RS-ALs of the three fish species (Lanes 1) showed SDS-PAGE patterns of typical Type I collagen. Little difference was observed among the patterns of the fractions A–C in the relative staining intensity of the bands of myofibrilliar proteins as myosin heavy chain (MHC) and actin (Ac). On the other hand, the staining intensity of the main band of collagenous components, α1(I), showing metachromasy was much more obvious in the fraction A than those in the fraction C, while the fraction B exhibited an intermediate level of staining intensity. These results reflected well the estimated collagen content of these fractions as shown in Table 2. In spite of the difference in the content of collagen, the electrophoretic pattern of the collagenous components was essentially similar among these fractions, suggesting the similarity of constituent collagen types among them. In the present SDS-PAGE analysis, we used Tris–HCl buffer containing neither urea nor 2-mercaptoethanol as a sample preparation buffer, although these reagents are usually used to solubilize the muscle proteins. This method showed an effect of promoting the predominant solubilization of collagen, resulting in the advantage of tracing the behavior of collagen.

Figs. 3 and 4 show the light microscopic images of the samples presently prepared from the three fish species. In the flesh, the connective tissues of myocommata, perimysium, and endomysium were observed to be arranged in an orderly manner. Partial disintegration was detected for these connective tissues and muscle fibers in the chopped meats, while the washed meats seemed to be swollen by the absorption of water during washing. As shown in Fig. 4, the relatively thick connective tissues, probably derived from myocommata, were frequently observed along with the thinner one in the fractions A and B. In contrast, the thick connective tissue was hardly observed in the fraction C, much thinner ones being exclusively contained in it. These results indicate that the thick connective tissues can be effectively removed by fractionation with a 30-mesh sieve.

Table 2
Moisture, protein and collagen contents of the surimi portions

Samples	Moisture (% wet sample)	Protein (% wet sample)	Collagen content		
			(% Wet sample)[a]	(% Dry sample)[b]	(% Protein)[b]
Red barracuda					
Flesh	72.6 ± 0.89	16.0 ± 0.33	0.49 ± 0.10	1.78	3.05
Fraction A	78.1 ± 0.63	14.7 ± 0.05	0.89 ± 0.11	4.08	6.05
Fraction B	78.4 ± 0.14	14.0 ± 0.19	0.35 ± 0.07	1.63	2.52
Fraction C	78.5 ± 0.14	14.0 ± 0.36	0.07 ± 0.01	0.30	0.46
Yellow sea bream					
Flesh	79.7 ± 0.19	15.9 ± 0.25	0.42 ± 0.06	2.07	2.65
Fraction A	83.1 ± 0.28	14.6 ± 0.18	0.80 ± 0.11	4.72	5.46
Fraction B	84.0 ± 0.10	14.3 ± 0.16	0.37 ± 0.06	2.33	2.61
Fraction C	84.2 ± 0.17	14.2 ± 0.09	0.07 ± 0.01	0.41	0.46
Spotted shark					
Flesh	77.1 ± 0.35	16.2 ± 0.34	0.73 ± 0.17	3.19	4.52
Fraction A	79.7 ± 0.29	20.0 ± 0.27	2.80 ± 0.06	13.8	14.0
Fraction B	82.6 ± 0.04	16.7 ± 0.16	1.24 ± 0.12	7.08	7.42
Fraction C	83.8 ± 0.24	14.9 ± 0.29	0.11 ± 0.01	0.66	0.71

[a] Significant differences ($P < 0.05$) were observed between fractions A–C for each fish species.
[b] Calculated from the present results for moisture, protein, or collagen content per wet weight of samples.

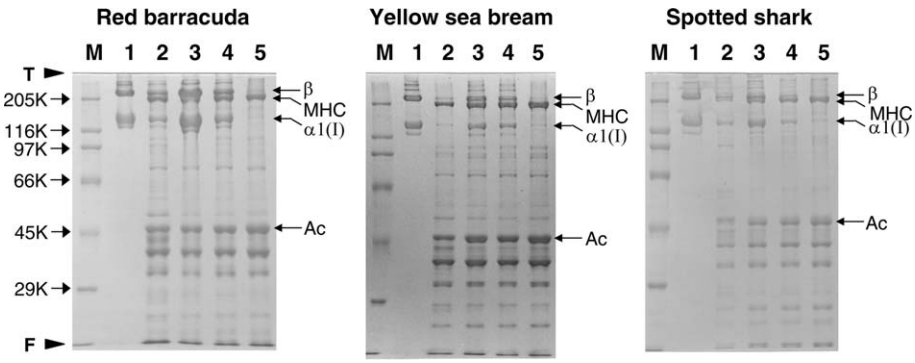

Fig. 2. SDS-PAGE analysis of the fractions A (lane 3), B (lane 4), and C (lane 5) from the three fish species, along with the RS-AL (lane 1) and flesh (lane 2). Lane M, molecular weight standard containing myosin from rabbit muscle (205 K), β-galactosidase from *Escherichia coli* (116 K), phosphorylase b from rabbit muscle (97 K), albumin from bovine serum (66 K), albumin from hen egg (45 K), and carbonic anhydrase from bovine erythrocytes (29 K).

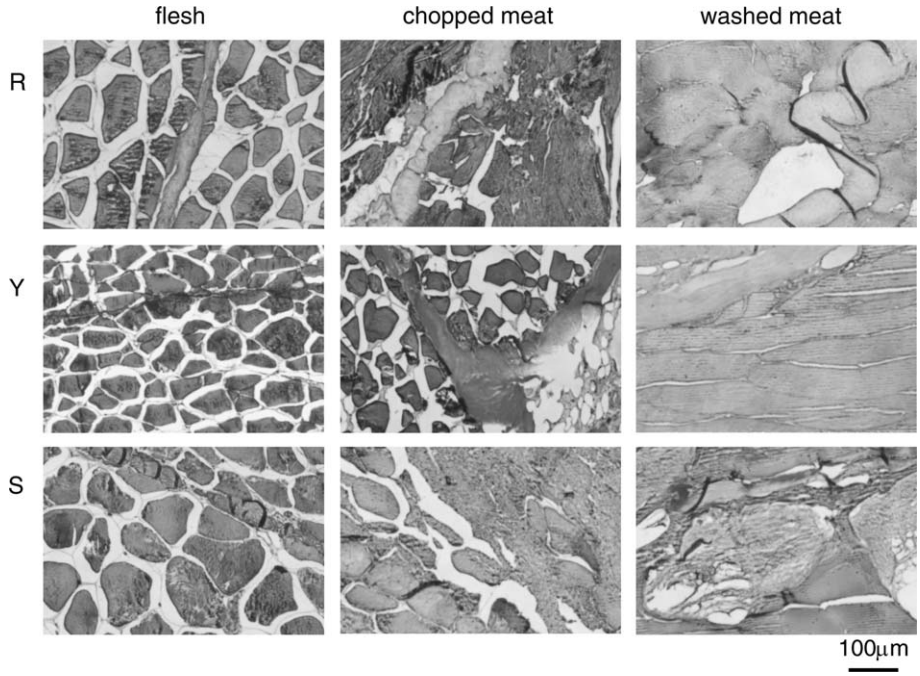

Fig. 3. Light microscopy of the flesh, chopped meat, and washed meat prepared from the three fish species. R, Y, and S, red barracuda, yellow sea bream, and spotted shark, respectively.

Collagen has received increased attention as an important proteinaceous component that contributes greatly to the textural development of raw or cooked meat of fish and shellfish (Hatae, Tobimatsu, Takeyama, & Matsumoto, 1986; Mizuta et al., 1994; Sato et al., 1997; Sato, Yoshinaka, Sato, & Shimizu, 1986). Therefore, it is considered that collagen or connective tissue may play some important roles also in the textural development of processed foods such as surimi-based products. According to Shiba (1994), flesh from a large size shark are usually fractionated in large-scale manufacturers by mechanical meat separator (hole size: 4 mmϕ) to be divided into the ''Suji'', which is the fraction rich in connective tissue, and the sieved surimi fraction. The product manufactured from the ''Suji'' fraction, also called ''Suji'', is famous as a special product of the Tokyo area. It has very firm texture in a refrigerated condition, but shows a unique soft texture when heated in soup due to the characteristics of gelatin (Shiba, 1994). It can be considered that the gelatin in the connective tissue in ''Suji'' may form a firm gel structure in the refrigerated condition and that it is disintegrated to sol in high temperature. Little fundamental information is, however, available concerning the variation of collagen content and connective tissue size in fish surimi fractionated with sieves or other mechanical meat separators. In the present study, the surimi from the three fish species were fractionated into two fractions (A and C) by sieving with stainless steel sieve, showing a marked difference in their collagen content and connective tissue size. Therefore, the present study may represent the first model experiment

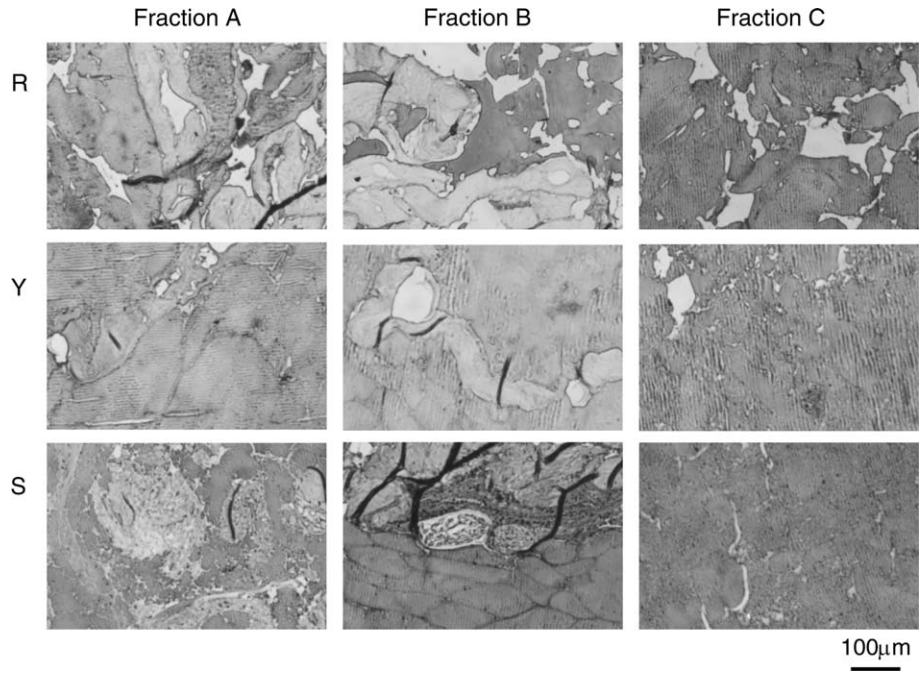

Fig. 4. Light microscopy of the fractions A–C prepared from the three fish species. R, Y, and S, the same as in Fig. 3.

on the behavior of connective tissue in the fractionation of fish surimi.

Further studies are now in progress to elucidate the roles of collagen or connective tissue in the textural development of set and heat-induced gels prepared from the fractionated surimi.

Acknowledgments

We wish to express our thanks to Dr. Y. Itoh, Department of Aquaculture, Faculty of Agriculture, Kochi University, Kochi, Japan, for his valuable advice related to the preparation of surimi.

References

Bremner, H. A., & Hallett, I. C. (1985). Muscle fiber – connective tissue junctions in the fish blue grenadier (*Macruronus novaezelandiae*). *Journal of Food Science, 50*, 975–980.

Duhamel, R. C. (1983). Differential staining of collagens and non-collagens with Coomassie Brilliant Blue G and R. *Collagen and its Related Research, 3*, 195–204.

Fairbanks, G., Steck, T. L., & Wallach, D. F. (1971). Electrophoretic analysis of the major polypeptides of the human erythrocyte membrane. *Biochemistry, 10*, 2606–2616.

Hatae, K., Tobimatsu, A., Takeyama, M., & Matsumoto, J. J. (1986). Contribution of the connective tissues on the texture difference of various fish species. *Bulletin of the Japanese Society of Scientific Fisheries, 52*, 2001–2007.

Kiernan, J. A. (1990). *Histological and histochemical methods: Theory and practice* (second ed.). Oxford: Pergamon Press.

Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (London), 227*, 680–685.

Micko, S., & Schlaepfer, W. W. (1978). Metachromasy of peripheral nerve collagen on polyacrylamide gel staining with Coomassie Brilliant Blue R-250. *Analytical Biochemistry, 88*, 566–572.

Mizuta, S., Yoshinaka, R., Sato, M., & Sakaguchi, M. (1994). Characterization of collagen in the muscle of several crustacean species in association with raw meat texture. *Fisheries Science, 60*, 323–328.

Sato, K., Ando, M., Kubota, S., Origasa, K., Kawase, H., Toyohara, H., et al. (1997). Involvement of type V collagen in softening of fish muscle during short-term chilled storage. *Journal of Agricultural and Food Chemistry, 45*, 343–348.

Sato, K., Yoshinaka, R., & Sato, M. (1989). Hydroxyproline content in the acid-soluble collagen from muscle of several fishes. *Nippon Suisan Gakkaishi, 55*(8), 1467.

Sato, K., Yoshinaka, R., Sato, M., & Shimizu, Y. (1986). Collagen content in the muscle of fishes in association with their swimming movement and meat texture. *Bulletin of the Japanese Society of Scientific Fisheries, 52*, 1595–1600.

Shiba, M. (1994). Utilization of shark muscle and surimi-based products. In *Kamaboko Nenkan* (The yearbook of surimi-based products) (pp. 250–261). Tokyo: Nihon Shokuhin Keizaisha.

Woessner, J. F. (1961). The determination of hydroxyproline in tissue and protein samples containing small proportion of this imino acid. *Archives of Biochemistry and Biophysics, 93*, 440–447.

Yata, M., Yoshida, C., Fujisawa, S., Mizuta, S., & Yoshinaka, R. (2001). Identification and characterization of molecular species of collagen in fish skin. *Journal of Food Science, 66*(2), 247–251.

Yoshinaka, R., Sato, M., Sato, S., Itoh, Y., Hujita, M., & Ikeda, S. (1985). Constituent proteins of muscle stromata from carp and Japanese mackerel. *Bulletin of the Japanese Society of Scientific Fisheries, 51*(7), 1163–1168.