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## Effect of Heat-Treatment on the Solubility and Viscosity of Salt-Extracted Protein from Mature Chum Salmon *Oncorhynchus keta*

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### Abstract

The effect of heat-treatment on the solubility and viscosity of salt-extracted protein of spawning chum salmon (skin color: dark) caught upstream was compared with those of pre-spawning chum salmon (skin color: bright) caught in coastal seas.

Proteins extracted from chum salmon muscle with 50 mM phosphate buffer (pH 7.0) containing various concentrations of NaCl were heated for 15 min at 20 to 100°C. The solubility of the sarcoplasmic protein fraction markedly changed during heating above 40°C. The heat-coagulability of the sarcoplasmic protein fractions from spawning salmon were not greater than that of pre-spawning salmon. For the 30-40°C treatment, total protein fractions extracted with 0.5 M salt from spawning salmon were more viscous than that from pre-spawning salmon. After heat-treatment above 40°C, the protein solutions showed a sudden decrease in viscosity.

### Introduction

Many of the chum salmon *Oncorhynchus keta* which have migrated for spawning are known to be of low quality as a food resource.<sup>1)</sup> For the effective utilization of mature chum salmon, it is necessary to understand various functional properties of its muscle or protein. Those functional properties might have changed with the physiological stage concerned with maturation.

Previously, Kawai et al.<sup>2,3)</sup>, Kaneko and Kawai<sup>4)</sup> reported that heat-coagulability, emulsifying properties and salt-extractability of muscle protein of spawning salmon were different from those of pre-spawning salmon. They have pointed out that this differentiation might affect the quality of the food manufactured from the spawning salmon using an emulsifying or heat-gelation process. In this regard, textural changes of chum salmon have been observed with the sexual maturation for heated<sup>5)</sup> and canned<sup>6)</sup> muscles.

The purpose of this paper is to explore the effect of heat-treatment on the solubility and viscosity as functional properties of salt-extracted protein from mature chum salmon muscle.

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## Materials and Methods

### *Fish*

Pre-spawning chum salmon (skin color: bright) was caught in a set-net in the coastal sea off Moheji, Kamiiso-cho, Hokkaido, and spawning chum salmon (skin color: dark) was caught in the Moheji River, Hokkaido. Ordinary muscle from the dorsal part of each fish from each group of salmon was collected, mixed and ground with a mincer. The prepared mince was then stored at  $-60^{\circ}\text{C}$  before use.

### *Proximate composition*

The moisture content was determined by the  $105^{\circ}\text{C}$ -drying method. Lipid content was determined by the method of Folch et al.<sup>7)</sup> Protein was calculated using the factor 6.25 from nitrogen content determined by the Kjeldahl method.

### *Salt-extractability*

The minced muscle was homogenized with a 9-fold volume of 50 mM sodium phosphate buffer (pH 7.0) containing various concentrations of NaCl. The homogenate was left for 2 h at  $4^{\circ}\text{C}$  and then centrifuged at  $10,000\times g$  for 15 min. Protein concentration of the supernatant was determined by the biuret method of Gornall et al.<sup>8)</sup> using bovine serum albumin (fraction V) as a standard.

### *Solubility*

The salt-extracted protein solution was adjusted to 5 mg/ml of concentration and heat-treated at various temperatures of 20 to  $100^{\circ}\text{C}$  for 15 min, then centrifuged as described above. The solubility was expressed as a percentage of protein concentration of supernatant after heating against that of unheated samples.

### *Viscosity*

Viscosity was measured at  $20^{\circ}\text{C}$  using a rotational viscometer Model BL-HM (Tokyo Keiki). Because muscle protein solution showed a non-Newtonian flow and very wide range of viscosity in this work, viscosity was expressed as a non-Newtonian coefficient of viscosity,  $\mu$  in the flow equation,  $s = \mu D^n$ , where  $s$ ,  $D$  and  $n$  were shear stress, shear rate and non-Newtonian index of viscosity, respectively.

## Results and Discussion

The biological and chemical characteristics of pre-spawning and spawning salmon used in this work are presented in Table 1.

Figure 1 shows the relationship between salt concentration and protein extractability. For both salmon muscle, protein extractability increased in the 0.2–0.4 M salt concentration and reached a maximum at 0.4–0.5 M salt concentration. Proteins extracted from the extractant of 0.2 M or lower concentrations were considered to be composed of mainly sarcoplasmic proteins, while proteins extracted from the extractant of 0.4 M or higher concentrations were composed of both sarcoplasmic and myofibrillar proteins, so referred to as total protein. The amount of sarcoplasmic and myofibrillar proteins extracted from spawning salmon tended to be less than that of pre-spawning salmon. These results show the same tendency as in a

Table 1. Biological characteristics of chum salmon and proximate composition of combined muscle mince.

	Pre-spawning (n=7)	Spawning (n=10)
Biological characteristics		
Fork length (cm)	70.5±3.8*	64.0±4.0*
Body weight (g)	3,900±610	2,790±570
Liver weight (g)	70±15	61±15
Hepatosomatic index	1.79±0.25	2.20±0.47
Gonad (milt) weight (g)	180±42	150±52
Gonadosomatic index	4.64±1.09	5.34±1.66
Proximate composition (g/100 g muscle)		
Moisture	73.97	78.79
Lipid	2.78	1.81
Protein	19.70	15.78
Extractive-N	0.52	0.53

\* Standard deviation

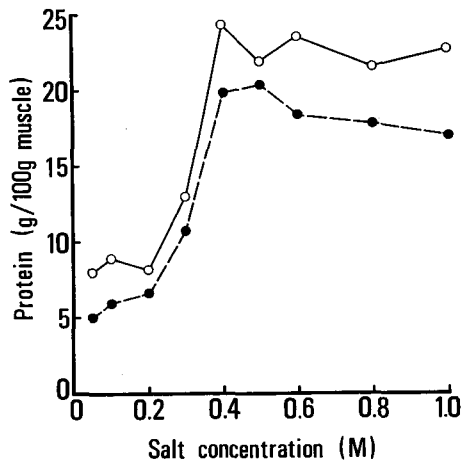


Fig. 1. Protein extractability with 50 mM sodium phosphate buffer (pH 7.0) containing various concentrations of NaCl from chum salmon muscle. Symbols: —○—, pre-spawning salmon; —●—, spawning salmon.

previous paper.<sup>3)</sup>

Viscosity of the homogenate prepared with each concentration of salt solution markedly increased due to solubilization of myofibrillar protein over 0.4 M salt concentration as shown in Fig. 2. Particularly, over 0.5 M salt concentration, the viscosity of spawning salmon was observed to be higher than that of pre-spawning salmon though the total solubilized protein and myofibrillar protein contents of spawning salmon were somewhat less in the homogenate.

Salt-extracted protein solution from chum salmon showed a non-Newtonian flow. In such a case, the viscosity is generally expressed as an apparent viscosity at

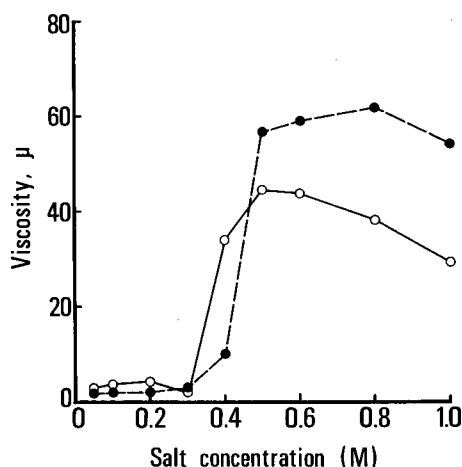


Fig. 2. The viscosity of muscle homogenate of chum salmon prepared with 50 mM sodium phosphate buffer (pH 7.0) containing various concentrations of NaCl.

The muscle was homogenized with a 9-fold volume of the buffer. The viscosity was shown as non-Newtonian coefficient of viscosity,  $\mu$ , in the flow equation,  $s = \mu D^n$  ( $s$ , shear stress;  $D$ , shear rate;  $n$ , non-Newtonian index of viscosity).

Symbols are the same as in Fig. 1.

a constant shear rate. In this work, however, the viscosity has been expressed as a non-Newtonian coefficient of flow because the viscosity had such a wide range that it could not be compared at a constant shear rate.

Changes in the solubility of the extract with various concentrations (0.05–1.0 M) of salt solution, i.e., soluble protein, by heating are as shown in Fig. 3.

For both pre-spawning and spawning salmon, sarcoplasmic proteins were soluble at 30°C or below, while the solubility decreased with heating above 35°C and reached a minimum at 60–80°C. The insolubility of sarcoplasmic proteins had a maximal value of about 77% at 60°C-heating for pre-spawning salmon while the value was 40–57% at 80°C-heating for spawning salmon.

The insolubility by heating of total protein fraction was maximal at 60°C and was proximately 15% or lower for both pre-spawning and spawning salmon. The total protein of spawning salmon had a slightly smaller insolubility than that of pre-spawning salmon.

Shimizu and Nishioka<sup>9)</sup> reported that the heat-coagulation of the mixture of actomyosin and sarcoplasmic protein from horse mackerel had become higher with an increase in the ratio of sarcoplasmic protein in the system. Figure 1 shows that the ratio of sarcoplasmic to myofibrillar protein in the total protein fraction was about 1 : 3 for spawning salmon and about 1 : 2 for pre-spawning salmon.

The results, i.e., that the heat-coagulability of spawning salmon was likely to be lower, agreed with the results of Kawai et al.<sup>2)</sup>, Kaneko and Kawai<sup>4)</sup>. They investigated the solubility changes according to heating temperature of protein of chum salmon with various quality grades associated with maturity caught in the east-coast of Hokkaido, and reported that the heat-coagulability of sarcoplasmic protein had been greater than of myofibrillar protein and decreased with the maturation.

Thus it can be surmised that heat-coagulability of the total protein fraction from mature chum salmon is mainly affected by two factors: the ratio of sarcoplasmic/myofibrillar protein and the coagulability of sarcoplasmic protein.

Figure 4 shows the relation between the viscosity and the heating temperature for total protein fractions extracted with 0.5 M salt solution. The protein solution

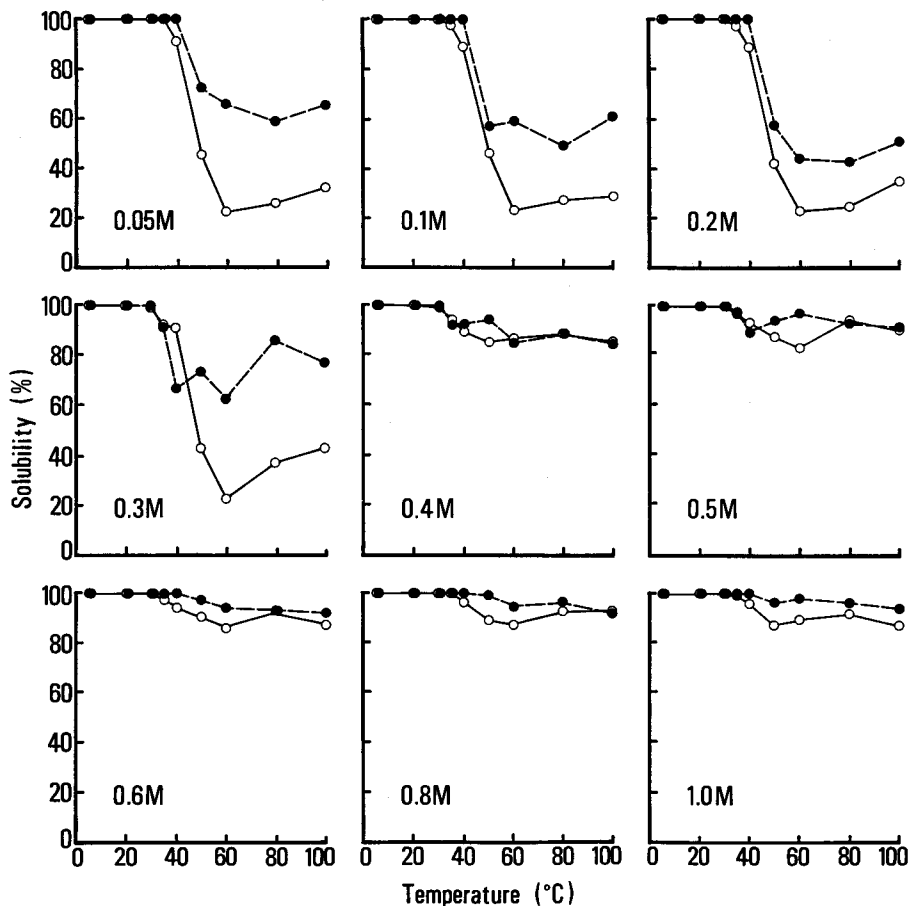


Fig. 3. Changes in the solubility of proteins extracted with 50 mM phosphate buffer (pH 7.0) containing various concentrations of NaCl from chum salmon muscle during heat-treatment.

Total salt concentrations of the extractants are given in the figure.

Symbols are the same as in Fig. 1.

at 30–40°C was very viscous, and the viscosity of protein from spawning salmon tended to be greater than that of pre-spawning salmon. Then the viscosity suddenly decreased above 40°C for both maturities. This was likely induced by apparent transformations in the protein molecular shape into globular, i.e., aggregated particle above 40°C from unfolded form at 30–40°C. Tsuji and Kawai<sup>5)</sup> suggested that the viscosity of the total protein fraction from mature chum salmon was affected by the quantitative and/or qualitative factors closely related with coagulation or aggregation by heating.

In general, to improve gel properties of kamaboko, water soluble sarcoplasmic protein is leached out from fish muscle, which somewhat inhibit the network formation of myofibrillar protein by coaggregation with the protein<sup>10)</sup>. Nishi and Kaneko<sup>11)</sup> reported that the leaching of spawning salmon muscle had not been so

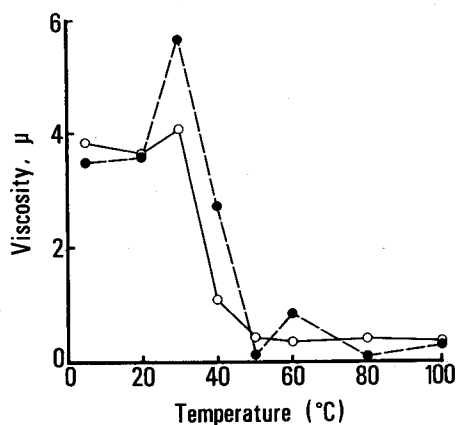


Fig. 4. Changes in the viscosity of protein solutions extracted with 0.45 M NaCl-50 mM sodium phosphate buffer (pH 7.0) from chum salmon muscle during heat-treatment. Protein concentration was 10 mg/ml. The viscosity was shown as in Fig. 2. Symbols are the same as in Fig. 1.

effective as pre-spawning salmon for kamaboko-gel forming. This might be interpretable by the lower heat-coagulability of sarcoplasmic protein of spawning salmon.

Thus, sexual maturation of chum salmon might affect the biochemical properties of its muscle protein so as to alter its heat-functional properties.

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