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Collagen content in farmed Atlantic salmon (*Salmo salar*, L.) and subsequent changes in solubility during storage on ice

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To elucidate whether collagen is an important factor for fish flesh quality, the collagen content and its changes in solubility during storage on ice in muscle of farmed Atlantic salmon (*Salmo salar*, L.) were measured. The contents of acid-soluble, pepsin-soluble and insoluble collagen in white muscle were determined in fresh fish muscle and at the end of 5, 10 and 15 days storage on ice. Total collagen was found to be 0.66% of fresh weight, with a relative distribution of 6% acid-soluble, 93% pepsin-soluble and 1% insoluble collagen. During storage on ice, a progressive change in solubility of muscle collagen was found. For insoluble collagen, significantly lower values were detected at day 15 compared to day 0. A minor, but even increase in acid-soluble collagen was found from day 0, while no changes were seen in pepsin-soluble collagen during storage. These results show that collagen fibres of farmed Atlantic salmon have a high solubility in acid and salt solutions and contain few cross-links. Some cleavage of intermolecular cross-links seems to occur during storage on ice.

INTRODUCTION

Collagen is one of the major constituents of intramuscular connective tissue. For textural properties of fish muscle, collagen plays an important role, and the softening of fish muscle during chilled storage on ice might be related to disintegration of collagen (Ando *et al.*, 1991*a,b*, 1992*a,b*, 1993; Chou, 1989; Cepeda *et al.*, 1990; Hallett and Bremner, 1988; Kensum and Norman, 1992). In rainbow trout (*Oncorhynchus mykiss*) muscle, the solubility of type V collagen is increased during storage, but not that of type I (Sato *et al.*, 1991, 1994). In chum salmon (*Oncorhynchus keta*) muscle cathepsins are found to be the enzymes responsible for the degradation of major structural proteins, both collagen and myofibrils (Mikami *et al.*, 1987; Yamashita and Konagaya, 1990, 1991, 1992). Weakening of connective tissue and disintegration of collagen fibrils have been studied in association with gaping (Lavéty *et al.*, 1988; Love, 1985). Gaping appears as slits or holes in the cut surface of the fillet and is claimed to be a quality problem in smoked salmon fillets.

Although much work has been done on collagen content and solubility of collagen in different fish species, more research on Atlantic salmon, focusing on flesh quality, is needed. The aim in the present work was to measure the collagen content in farmed Atlantic salmon (*Salmo salar*, L.) and to eludicate the solubility of collagen during storage on ice.

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MATERIALS AND METHODS

Materials

Atlantic salmon (*Salmo salar*, L.) were obtained from a commercial fish farm, Hydro Seafood, Bergen, on the west coast of Norway. Four fish with an average weight of 4.4 kg were transported on ice, and immediately prepared at the laboratory. The muscle from the dorsal part of the trunk was separated from skin and dark muscle and sliced into pieces. One piece was used immediately, and the remainder was stored in plastic bags on ice at 4°C until analyses were carried out on days 5, 10 and 15.

Preparation of collagen

All operations were performed in a cold room at 4°C. Acid-soluble (ASC), pepsin-soluble (PSC) and insoluble collagen fractions (ISC) were prepared by a method by

Sato *et al.* (1988) that involved preliminary extraction with cold 0.1 N NaOH solution to remove non-collagenous proteins. The alkali extraction included homogenizing of muscle with 10 vols (v/w) 0.1 N NaOH followed by centrifugation at 10 000 g for 20 min. The residue was treated with 20 vols NaOH solution, stirred overnight and centrifuged (10 000 g). The NaOH addition, followed by centrifugation, was repeated four times. The final precipitate was washed with distilled water. Thereafter, acid-soluble collagen was fractionated by adding 10 vols (v/w) 0.5 M acetic acid to the precipitate, stirred for 2 days, and centrifuged at 10 000 g for 20 min. Pepsin-soluble collagen was rendered soluble by limited digestion with porcine pepsin (Sigma, 2× crystallized) at an enzyme:substrate ratio of 1:20 (w/w) in 0.5 M acetic acid. The digestion was performed at 37°C for 2 days before centrifugation at 10 000 g for 20 min. The final insoluble matter was used for the insoluble collagen preparation.

Chemical analysis of hydroxyproline in ASC, PSC and ISC fractions

Hydroxyproline content, as determined according to a colorimetric method described in ISO (1978), is basically a method for determination of hydroxyproline content in meat and meat products. The first step in this method, which is to hydrolyse the crude sample to release hydroxyproline, was slightly modified to adjust the determination of hydroxyproline in the prepared sample solutions of ASC, PSC and ISC from fish. One ml sample from the prepared collagen fractions was treated with 1 ml 60% sulphuric acid. After hydrolysing over night (12 h), the samples were diluted to 50 ml to make the final concentration of hydroxyproline between 0.5 and 2.4 μg/ml. The later steps in the method were performed as described in ISO, 1978, where the principle is oxidation of hydroxyproline by chloramin-T, followed by addition of 4-dimethylaminobenzaldehyde, leading to a coloured complex which is measured photometrically at 560 nm. To convert the amount of hydroxyproline to collagen in salmon muscle, a factor of 11.42 was used (Sato *et al.*, 1991).

Statistics

The results are given with standard deviation, SD (Figs 1 and 2) and standard error of mean, SEM (Table 1). Analysis of variance, ANOVA, followed by Tukeys HSD test were used to calculate significant differences in the amounts of ASC, PSC and ISC versus storage time. Significant differences were assessed with a given level of $p < 0.01$. The development of ASC, PSC and ISC over time was tested by Pearson Product-Moment Correlation analyses. All statistics were performed using CSS Statistica (Statsoft, 1991, Tulsa, USA).

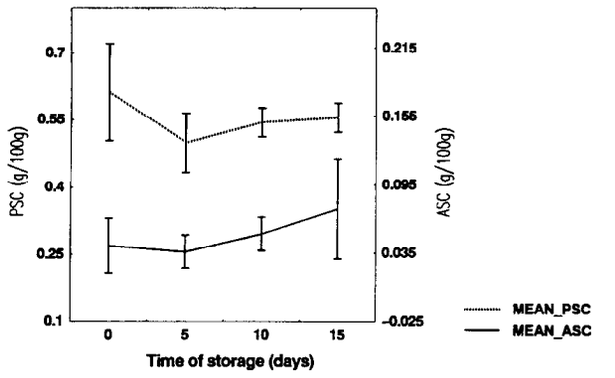

Fig. 1. Changes in solubility of acid-soluble (ASC) and pepsin-soluble (PSC) collagen fractions (mg/100 g) in muscle of farmed Atlantic salmon ($n = 4$) during 15 days storage on ice.

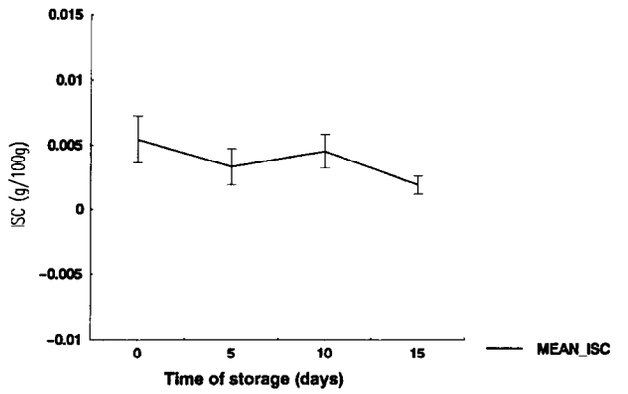

Fig. 2. Changes in solubility of the insoluble (ISC) collagen fraction (mg/100 g) in muscle of farmed Atlantic salmon ($n = 4$) during 15 days storage on ice.

RESULTS AND DISCUSSION

The results given in Table 1 show that the total collagen content in fresh farmed Atlantic salmon muscle amounted to 0.66% of wet weight (w.w.), which is quite low, but in agreement with results reported for other fish species (Kimura and Matsui, 1985; Sikorski *et al.*, 1984; Sato *et al.*, 1986*a*,*b*). A variation in total collagen from 0.34% (sardine, *Sardinops melanostictus*) to 2.19% (conger eel, *Conger myriaster*) of w.w. white muscle was found in an evaluation of 24 different fish species by Sato *et al.* (1986*b*). For red sea bream (*Pagrus major*) the total collagen content was 0.37% and for Japanese eel (*Anguilla japonicus*) 1.28% w.w. (Yoshinaka *et al.*, 1990). In the study of Sato *et al.* (1986*b*) it was found that sardine (*Sardinops melanostictus*), brook masu salmon (*Oncorhynchus masou masou*), argentine (*Glossanodon semifasciatus*), rainbow trout (*Oncorhynchus mykiss*) and horse mackerel (*Trachurus japonicus*), all with a low total muscle collagen content (0.34%–0.51%), had a tender meat vulnerable to gaping. The relationship between collagen content and texture was further confirmed by Hatae *et al.* (1986), who showed that a high collagen content resulted in a firm meat.

Table 1. Total collagen (g/100 g) and the relative distribution of collagen content (%) of acid-soluble (ASC), pepsin-soluble (PSC) and insoluble (ISC) collagen fractions of farmed Atlantic salmon ($n = 4$) during 15 days of storage

Fraction	Days 0	5	10	15	SEM pooled	Correlation % against time
Total (g/100 g)	0.66	0.54	0.6	0.63	0.036	
ASC (% of total)	6.51	6.7	8.5	11.3	1.73	$r^2 = 0.4857$, $p = 0.056$
PSC (% of total)	92.7	92.7	90.7	88.3	1.78	$r^2 = -0.4446$, $p = 0.084$
ISC (% of total[a,b])	0.84	0.62	0.74	0.3	0.11	$r^2 = -0.5949$, $p = 0.015$

[a]Significant difference between day 0 and day 15 ($p < 0.01$) with use of ANOVA.
[b]Significant difference of the development of %ISC against time ($p < 0.05$).

The relative distribution of concentrations of collagen fractions given in Table 1 were 6% for ASC, 93% for PSC, and 1% for ISC. This shows that almost all the collagen found in white muscle of farmed salmon, even before storage, was soluble in acid or salt solutions, which indicates that salmon, like other fish species, has a unique collagen with a high solubility in acid and salt solutions, the opposite of collagen found in mammalian meat (Sato, 1993).

Exogenous enzymes, such as pepsin, have been used routinely to cleave non-helical domains of collagen and consequently to solubilize collagen from tissues without cleavage of the triple helix domain (Sato, 1993). Addition of pepsin in the present study, which solubilized 93% of the total amount of collagen (Fig. 1) and left only 1% insoluble collagen, indicates that there are few cross-links of collagen in salmon muscle. According to Montero and Borderias (1990*a*; Montero and Borderias, 1990*b*), few cross-links indicate a low shear strength. The distribution of soluble and insoluble collagen in fish muscle reported in other studies varies from species to species. For Japanese eel (*Anguilla japonicus*), the dorsal part of the trunk muscle contained 17% soluble collagen and 83% insoluble collagen, while red sea bream contained 73% soluble and 27% insoluble collagen, respectively (Yoshinaka *et al.*, 1987). It is suggested that these differences in muscle collagen solubility are related to the flexibility of the body. Based on this, we conclude that the high amount of soluble collagen, in farmed salmon muscle, results in a relatively soft body.

During storage, there was a progressive change in the solubility of muscle collagen. Insoluble collagen decreased gradually, with a significant difference found between days 0 and 15. After 15 days storage on ice, the insoluble collagen fraction decreased to approximately one third of the value achieved at day 0, from 0.0054 to 0.0019 g/100 g (Fig. 2). A positive correlation was found for the development of acid-soluble collagen over time ($r^2 = 0.4857$), and the change was close to, but not significant at $p < 0.05$ ($p = 0.056$). No significant changes were seen for acid-soluble collagen, and no increase in pepsin-soluble collagen was measured during storage (Table 1). The decrease of insoluble collagen during storage was probably a result of environmental changes in the salmon muscle post-mortem, where enzymes like collagenases, neutral proteinases and acid proteinases had cleaved parts of the triple helix (Pearson and Young, 1989). Sato *et al.* (1987) demonstrated the presence of proteases in fish muscle, which degraded non-helical regions of collagen and solubilized collagen *in vitro*. Further, it has been reported that cathepsin L and serine proteases are capable of hydrolysing major muscle structural proteins such as collagen (Sato *et al.*, 1994; Yamashita and Konagaya, 1991), while the extracellular matrix collagenases are active against collagen of type I, IV and V and are regarded as initiators of breakdown (Bremner, 1992).

Raw fish meat from most fish species softens rapidly during chilled storage, which histological examinations have shown to be caused by disintegration of collagen (Sato *et al.*, 1991). Therefore, post-mortem changes of connective tissue constituents rather than degradation of myofibrillar proteins, such as myosin, connectin, and actin, might be responsible for the rapid softening of raw fish flesh during chilled storage (Sato *et al.*, 1994). The results from the present study support this theory.

Quality problems with farmed salmon have arisen during the last few years, especially after the introduction of high energy feeds at the beginning of the 1990s. Feeding these energy-dense diets with a fat content above 30% has resulted in enhanced growth rates of salmonids, which in turn has been linked to poor flesh quality (Hillestad and Johnsen, 1994; Sheehan *et al.*, 1996). The fish flesh has become soft with a relatively high fat content, and complaints about the fish flesh gaping have occurred. After smoking, these textural problems might result in difficulties with slicing. Also, farmed salmon are often stored for several days before reaching the smokeries, which may result in an increased post-mortem softening and occurrence of gaping. To conclude, the quality defects in farmed Atlantic salmon are quite complex. One of the reasons may be the measured changes in muscle collagen structure during chilled storage. However, further studies should include environmental parameters during rearing, such as feed and growth rate, as well as slaughter technique effects and storage parameters, in the hope of good quality control in the future.

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