Postmortem Changes of Cultivated Atlantic Salmon and Their Effects on Salt Uptake

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ABSTRACT

Rigor mortis of cultivated Atlantic salmon (*Salmo salar*) began to set in 8h after death and was fully resolved 60-70h after death during storage at 0°C. Maximum muscle contraction was observed 24–30h after death. ATP content decreased from 7.25 to 0.14 to 0.09 μmol/g fish from pre-rigor mortis to in-rigor mortis to post-rigor mortis state. The inosine and hypoxanthine contents increased from 0 to 1.20 to 4.06 μmol/g fish and from 0.08 to 0.33 to 0.84 μmol/g fish during 60h storage, respectively, during 60h of storage at 0°C. Postmortem changes affected salt uptake. The equilibrium salt concentrations of pre-rigor, in-rigor and post-rigor mortis salmon were 0.53, 0.66 and 0.75 g/g salt-free solids, respectively, in a 20% (w/v) sodium chloride solution at 10°C.

Key Words: salmon, K value, muscle, postmortem, rigor index

INTRODUCTION

RIGOR MORTIS IS ONE OF THE MOST PROMINENT CHANGES IN muscle occurring soon after death. When fish are killed while relaxed, creatine phosphate is degraded prior to the breakdown of adenosine triphosphate (ATP) (Iwamoto et al., 1988). When the creatine phosphate and ATP reach about the same concentration as ATP, ATP content begins to decrease (Watabe et al., 1991) and rigor mortis starts (Iwamoto et al., 1987). The muscle enters a full-rigor mortis as ATP decreases to about 1 μmol/g. Rigor mortis occurs when crossbridge cycling between myosin and actin in myofibrils ceases, and permanent actin and myosin linkages are formed (Pate and Brokaw, 1980). Rigor mortis is resolved after some time. Possible causes of postmortem tenderization include a weakening of Z-discs of myofibrils (Hultin, 1984; Seki and Tsuchiya, 1991), a degradation of connective tissue (Seki and Watanabe, 1984; Ando et al., 1993), or a weakening of myosin-actin junctions (Yamanoue and Takahashi, 1988).

Several methods for evaluating rigor mortis have been reported. Direct measurements are based on the changes of physical and mechanical properties such as rigidity/rigor index (Bito et al., 1983), shear strength (Montero and Borderias, 1990), and isometric muscle strength (Nakayama et al., 1992). Berg et al. (1997) evaluated rigor mortis development of farmed Atlantic salmon using a mechanical rigorometer and low-frequency vibration method (LFV). They classified the rigor state by using the LFV-method combined with neural network analysis. They concluded that assessment by the rigorometer was not suitable for accurate evaluation but it might be used for following the rigor progress in individual fish. Indirect methods measure the changes of pH and concentration of the products of autolysis during the course of rigor mortis, as it is associated with the breakdown of ATP. Saito et al. (1959) suggested that the amounts and ratios of certain nucleotide degradation products might be used as indicators of the storage age or freshness of fish. The original method for determining those products was based on using ion exchange chromatography to isolate inosine (HxR) and hypoxanthine (Hx) from acid soluble nucleotides. Saito et al. (1959) also suggested the use of the coefficient $K = 100\ B/A$, where A is the total absorbance at 254 nm of a perchloric acid extract of the fish sample and B is the sum of the absorbance of inosine and hypoxanthine at the same wavelength. Several methods have been applied for the quantitative determination of ATP catabolites (Martin et al., 1978). High-performance liquid chromatography (HPLC) has mostly replaced the time-consuming ion exchange chromatography method for those analyses (Ryder, 1985; Cann-Moisan et al., 1989). Very rapid methods of determining the K value have also been reported. Karube et al. (1984) used a biosensor that could determine the K value in a few minutes, also Negishi and Karube (1989) developed a rapid test paper method.

Salting is widely used for preserving fish, sometimes in combination with drying or smoking. Harvesting and storage conditions affect postmortem changes of fish quality (Hultin, 1985). Rigor mortis has been hypothesized to have a major effect on salting processes, but little has been published on the quantitative influence of rigor mortis on the salting effects of Atlantic salmon. Our objectives were: (1) to examine rigor mortis and ATP degradation in cultivated Atlantic salmon during storage at 0°C; and (2) to determine the effects of rigor mortis on equilibrium salt concentrations in the muscle.

MATERIALS & METHODS

ATLANTIC SALMON (*SALMO SALAR*) OF COMMERCIAL ORIGIN, weighing 2.1 ±0.5 kg (mean±SD, n=17), were cultivated in aerated sea water at 5–9°C with dissolved oxygen levels of 80–110% saturation. They were fed daily on a diet of Fundy Choice feed #8 (Corey Mills Ltd., Fredericton, NB). The fish were starved for 2 days prior to harvest. They were caught with a small net and immediately killed by a sharp blow to the head and then bled at the gills. They were laid on ice (0°C) and covered by ice on the top in a cooler and transported to a chill room (3±1°C) within 30 min. At various time intervals, fish samples were taken out to examine rigor mortis progress.

Rigor index

The rigor index was measured according to Bito et al. (1983) and used as a parameter to determine the stage of rigor mortis. In measuring the rigor index, the upper half of a whole fish was placed on one side on a horizontal table surface with the other half (tail part) suspended off the edge. At selected time intervals, the vertical distance (L) between the base of the caudal fin and the table surface was measured, and the rigor index was calculated as:

$$\text{Rigor index (\%)} = [(L_o - L)/L_o] \times 100\% \quad (1)$$

where, L_o is the vertical distance between the base of the caudal fin and the table surface measured immediately after the death.

In the salting experiments, rigor index was used to monitor the development of rigor mortis. The rigor mortis states of the fish were classified according to the following criteria: (1) pre-rigor mortis: no stiffening, full movement of muscle (rigor index ≤ 10%); (2) in-rigor mortis: fully stiffened (80% ≤ rigor index ≤ 100%); (3) post-

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rigor mortis: the state when rigor index ≤ 10%. The in-rigor mortis period contained in the onset period, which was from the first detectable stiffening through full development of stiffening. The onset period was indicated by 10% < rigor index ≤100%.

Sample preparation

At a specific rigor mortis state as indicated by the rigor index, each fish was cut into two fillets and skinned. The dorsal muscle of one fillet was homogenized for the determination of nucleotides and total solids. The dorsal region of the other fillet was cut into 10 slices with dimensions 30 mm × 30 mm × (4–5) mm by a slicer (Model 610, Hobart Canada, North York, ON). These samples were used for brining. All chemical analyses were performed in triplicate (i.e., samples from 3 different fish were analyzed).

Nucleotide analyses

At each rigor mortis state, 25g of dorsal muscle were blended with 125 mL of 0.6M perchloric acid at 0°C for 1 min in a Multi-Mixer (Model MM-1B, Lourdes Instrument Co., Brooklyn, NY) and filtered using Whatman #1 filter paper. Filtrate (10 mL) was neutralized to pH 6.5–6.8 with 1M KOH. The solution was diluted to 20 mL prior to storage at −35°C until the analysis was performed. Ryder's (1985) HPLC method was used with a Waters HPLC system (Waters Associates Inc., Milford, MA) equipped with two Model 6000 A pumps, a WISP 710B autosampler, a Model 450 variable-wavelength detector, a Model 720 System Controller and Data Module. The separation was achieved using a C-1 μBondapak reverse-phase column (3.9 mm × 300 mm). The mobile phase was 0.1M potassium phosphate with pH 7.0. Other chromatographic conditions were: flow rate, 1 mL/min; injection volume, 5 μL; column temperature, 30°C; UV detection at 254 nm; and run time, 40 min. The nucleotides were identified by their relative retention times and externally quantified using standards obtained from Sigma Chemical Company (St. Louis, MO).

K values

The freshness of the muscle was judged from the K values, defined as follows (Saito et al., 1959):

$$\text{K value (\%)} = \frac{[HxR] + [Hx]}{[ATP]+[ADP]+[AMP]+[IMP]+[HxR]+[Hx]} \times 100\% \quad (2)$$

where each compound is expressed as molar concentration. ADP=adenosine diphosphate, AMP=adenosine monophosphate, and IMP=inosine monophosphate. As ATP, ADP, AMP and IMP are converted to HxR and Hx after the death of fish, the K values were inversely correlated to the freshness of the fish.

Brining procedure

Each sample slice, prepared as described, was soaked in a separate beaker containing 200 mL 20% (w/v) sodium chloride solution at 10±0.5°C. The brining temperature was set at 10°C because this is the temperature commonly used in the smoked salmon industry (Truelstrup-Hansen et al., 1995). At various time intervals, a sample was taken out and excess solution on the surface was removed with an absorbent paper tissue. Samples were then stored at −35°C until the salt contents were determined. To determine the time required to reach equilibrium salt concentration, the salmon slices were soaked at time intervals of 6, 12, 24, 36 and 48h. Pre-rigor mortis and post-rigor mortis salmon were used for these experiments because they represent the two extreme postmortem conditions. For in-rigor salmon muscle, only the salt concentration in the sample soaked for 48h was investigated.

Sodium chloride and total solid

The sodium chloride content of the fish samples was determined by the AOAC Official Method 937.06 (AOAC, 1995). The salt concentration in the muscle was expressed as g salt/g salt-free solids (SFS). Quantity of total solids was determined by drying 10g homogenized salmon sample at 103±1°C for 22–24h to constant weight.

Statistical analysis

The SAS® procedure GLM (general linear models) (SAS Institute Inc., 1988) was used for the analysis of variance on the HP-UNIX® (HP 700 series) mainframe computer. The Duncan's multiple range tests were applied for rigor index, ATP and its breakdown products and equilibrium salt concentrations. A significance level of $P<0.05$ was chosen. Values were reported as mean±SD.

RESULTS & DISCUSSION

Rigor index

The rigor mortis progress of Atlantic salmon was investigated by examining both physical and biochemical changes during postmortem storage. The physical changes of Atlantic salmon when stored in ice were determined and expressed as rigor index (Fig. 1). Rigor mortis began to set in about 8h after death and was fully resolved 60–70h after death. Maximum muscle contraction was observed 24–30h after death. This confirmed reported rigor mortis development of unstressed Atlantic salmon (Berg et al., 1997). The standard deviations were relatively large during onset and resolution of rigor mortis. This may have been caused by the variation of fish size (average 1.875 kg, SD=0.192 kg) and the stress of each fish upon capture. Analysis of variance showed that rigor index was affected by the time ($P<0.05$), as expected, but not different ($P>0.05$) among the fish studied.

ATP and its breakdown products

ATP and its breakdown products, ADP, AMP, IMP, HxR and Hx, were determined as additional indicators of rigor mortis. The biochemical changes of postmortem Atlantic salmon during storage at 0°C were investigated by examining ATP and its breakdown products at pre-rigor mortis, in-rigor mortis and post-rigor mortis state. Average amounts of the nucleotides detected in salmon muscle during storage at 0°C are compared (Table 1). There was considerable fish to fish variation in the ATP, ADP, AMP, and IMP concentrations at the pre-rigor mortis state. The ATP content in the muscle decreased from 7.25 to 0.14 to 0.09 μmol/g fish from pre-rigor mortis to in-rigor mortis to post-rigor mortis state because of its degradation af-

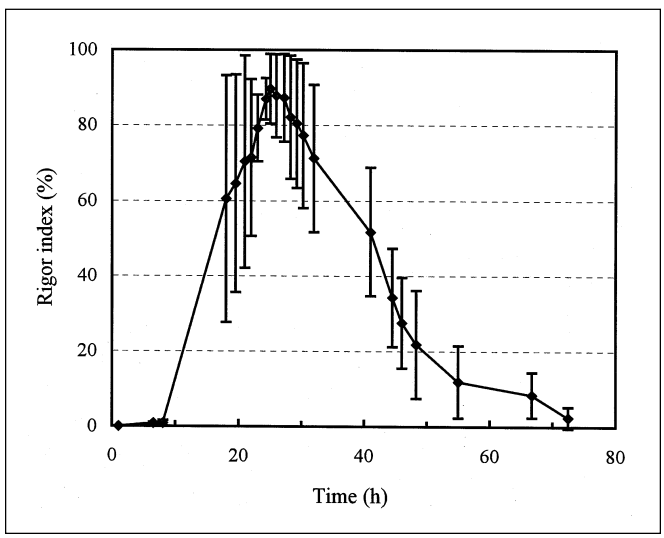

Fig. 1—Rigor index histories of Atlantic salmon stored at 0°C. The curve shows the mean of four replicated tests and the error bars show standard deviations.

Table 1—Changes of ATP and its breakdown products in Atlantic salmon muscle[a]

Rigor mortis state	ATP (μmol/g)	ADP (μmol/g)	AMP (μmol/g)	IMP (μmol/g)	HxR (μmol/g)	Hx (μmol/g)	K value (%)
Pre-rigor	7.25 ± 2.69	0.41 ± 0.58	0.12 ± 0.17	4.16 ± 3.22	0.00 ± 0.00	0.08 ± 0.03	0.69 ± 0.26
In-rigor	0.14 ± 0.12	0.37 ± 0.10	0.25 ± 0.12	12.09 ± 0.75	1.20 ± 0.24	0.33 ± 0.12	10.60 ± 2.08
Post-rigor	0.09 ± 0.07	0.17 ± 0.07	0.19 ± 0.08	7.16 ± 0.63	4.06 ± 1.31	0.84 ± 0.23	41.09 ± 7.72

[a]Each value is the mean of three replicates.

ter death (Table 1). Iwamoto et al. (1987) reported that ATP in plaice (*Paralichthys olivaceus*) decreased to <1 μmol/g fish when the rigor index was 100%. This was also true for Atlantic salmon. In our results, ADP, AMP and IMP were found in all samples of pre-rigor salmon, but no HxR and only traces of Hx were detected. HxR and Hx contents increased from 0 to 4.36 μmol/g fish and 0.08 to 0.85 μmol/g fish from pre-rigor mortis to post-rigor mortis, respectively, during storage. The accumulation of HxR confirmed results reported by Surette et al. (1988), who suggested that in Atlantic cod the subsequent production of inosine was the rate-limiting factor in the ultimate formation of hypoxanthine, xanthine, and uric acid.

The K value, often used to express freshness of fish, was calculated (Table 1). The K values increased from 0.7% to 10.6% to 41.1% from pre-rigor mortis to in-rigor mortis to post-rigor mortis state, which indicated decreasing freshness. Our results were in the same range as those of Erikson et al. (1997), who reported that K values of unstressed farmed Atlantic salmon (*Salmo salar*) were 12% and 34% after 1 day and 3 days postmortem, respectively. Rigor index indicated the biochemical and physical changes and was chosen as an indicator of rigor. It could also be used as an indicator of rigor mortis of Atlantic salmon in the fish processing industry. K value development during ice storage of salmonids has been reported by Uchiyama (1988), Boyle et al. (1991), Luong et al. (1991), and Hattula and Kiesvaara (1992).

Equilibrium salt concentration

Average salt concentrations in salmon slices [30 mm × 30 mm × (4–5) mm] during salting in 20% (w/v) brine at 10°C were plotted (Fig. 2). Data for only pre-rigor mortis and post-rigor states were obtained, because our preliminary tests showed no difference in salt uptake between in-rigor mortis and post-rigor mortis Atlantic salmon fillet. Salt content in salmon slices increased sharply during the first 10h of soaking. It then approached a plateau after 25h soaking. The salt concentration in fish at salting time of 36h was> 99% of the corresponding value at 48h. Therefore, 48h was considered sufficient for the muscle salt content to reach the equilibrium concentration. The in-rigor mortis fish slices were then soaked in 20% (w/v) brine at 10°C for 48h with three replicates in order to determine their equilibrium salt concentration (Table 2).

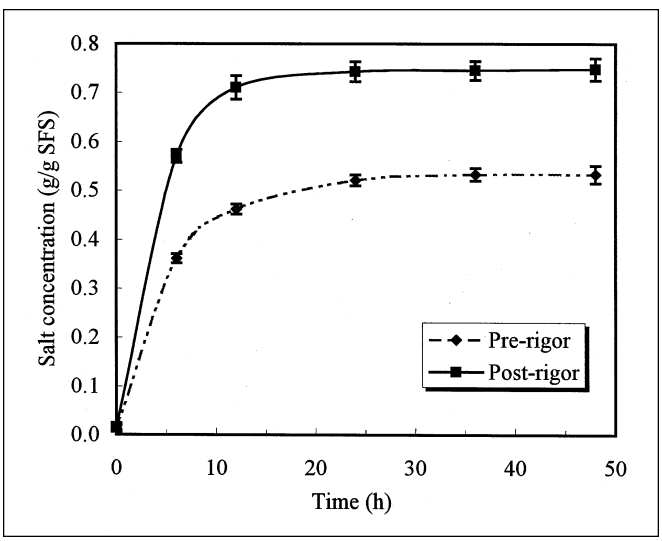

Fig. 2—Average salt concentration in salmon slices during salting in 20% (w/v) brine at 10°C.

Table 2—Equilibrium salt concentration in Atlantic salmon muscle in 20% (w/v) brine at 10°C

Rigor mortis state	Equilibrium salt conc (g/g SFS)	
	Mean	SD
Pre-rigor	0.53[a]	0.07
In-rigor	0.66[b]	0.07
Post-rigor	0.75[b]	0.02

[a,b]Each value is the mean of three replicated tests; mean values followed by different letter superscripts are significantly different (P<0.05).

The equilibrium salt concentration in Atlantic salmon muscle in 20% (w/v) brine was lower for pre-rigor mortis fish than for either in-rigor mortis or post-rigor mortis fish (Table 2). The equilibrium salt concentration in in-rigor and post-rigor mortis salmon tissue were no significantly different. Published values for the equilibrium salt concentration of cures with excess salt was generally between 0.75 and 0.80 g/g SFS (Peters, 1971). Because a lower brine concentration was used in our research, we observed slightly lower equilibrium salt concentration than those reported by Peters (1971). The equilibrium salt concentration of fresh swordfish in 3.42 mol/L (i.e., 20% w/v) brine at 10°C was 0.70 g/g SFS when interpolated from the reported data of Del Valle and Nickerson (1967). This was near the equilibrium salt concentration of in-rigor and post-rigor mortis salmon determined in our research. The effect of rigor mortis on the equilibrium salt concentration in salmon has not been reported. A possible explanation for the higher equilibrium concentrations for in-rigor and post-rigor mortis fish was the degradation of cellular structures by enzymes. Perhaps in pre-rigor mortis fish, the equilibrium salt concentration was lower due to the intact membranes that maintained a certain level of osmotic pressure to balance the differential solution concentrations in and outside of the cells. For in-rigor and post-rigor mortis fish, the cells are broken down and so the equilibrium salt concentration is reached in both the aqueous phase surrounding the cells and as well as inside the cells, i.e., a larger fraction of the slice volume (Schwartzberg and Chao, 1982).

CONCLUSIONS

ANALYSIS OF ATP AND ITS BREAKDOWN PRODUCTS USING HPLC confirmed results of rigor index determinations in indicating the postmortem changes and, thus, the decrease of freshness of Atlantic salmon. Rigor mortis of Atlantic salmon began to set about 8h after death and was fully resolved 60–70h after death, with maximum contraction occurring between 24 and 30h after death when stored at 0°C. Initial postmortem changes of Atlantic salmon after death greatly influenced the rate of salt uptake and final salt concentration during salting. The equilibrium salt concentration of pre-rigor salmon fillet was much lower than those in-rigor and post-rigor mortis salmon fillets in the same brine solution. Therefore, higher salt concentration brine should be used for pre-rigor mortis salmon salting than for in-rigor or post-rigor mortis Atlantic salmon if similar levels of equilibrium salt concentration are desired.

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