



Effect of Temperature Fluctuation on Quality of Frozen Atlantic Salmon (*Salmo Salar*) Fillet

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Abstract: Cold chain plays a vital role to guarantee food quality and safety during production, storage, transportation and retail operations by keeping product temperature within an acceptable range. Nevertheless, temperature fluctuation occurs commonly in the cold chain leading to complete or partial thawing and re-freezing of frozen products. Little is known about the destructive effect of temperature fluctuation, particularly concerning low levels of fluctuations. The objective of this experiment was conducted to understand some quality-related changes occurring due to temperature fluctuation in the cold chain of Atlantic salmon (*Salmo salar*). Changes in drip loss, pH and the fractional space between muscle fibers were studied in fillet stored at four different levels of temperature fluctuation. Fillets stored in low fluctuation temperatures levels (NF and LF) showed significantly lower drip loss ($p < 0.05$), higher pH and smaller area fraction of the space between muscle fibers ($p < 0.05$) than fillet stored at the high level of temperature fluctuation (HF and VF). The finding showed the destructive effect of temperature fluctuation even at lower levels of temperature fluctuation.

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INTRODUCTION

Fish is one of the most perishable products due to its biological composition. Several technologies of food preservation have been in use to extend the shelf life of fish and fishery products. Preservation using freezing technology is one of the popular methods used to extend the shelf life of fish for the long term without a considerable effect on quality. Preservation by freezing works in two ways: through converting the active liquid water to ice and through lowering the rate of chemical reaction because of the lowered temperature.

The global fish market relies on this technology to safely distribute products throughout the world market. Once the required procedures such as freezing, storing and thawing are followed accordingly, it is possible to keep the organoleptic and nutritional quality of the end product^[1]. For instance, the color, taste and texture of a fish stored for three months at freezing temperature were not differentiable from fresh fish, according to Cappeln *et al.*^[2] and Nielsen and Jessen^[3].

Several factors including storage temperature, freezing rate and temperature fluctuation may affect the quality of frozen food products^[4]. Temperature fluctuation

occurs frequently in cold chains leading to complete or partial thawing and re-freezing of the frozen product^[5]. This could result in a significant loss of textural quality, nutritional value and functional property^[6]. Even, low degrees of temperature fluctuation resulting in partial-thawing of a product has shown to affect the size of ice crystals which might lead to loss of quality of end product^[5, 7]. Thus, despite the advantage of freezing technology in maintaining the quality of fish and fishery products for an extended period, interruption of the cold chain leading to temperature fluctuation is being concerning the industry and consumers^[8, 1].

Studying the effect of temperature fluctuation on the quality of fish muscle is, therefore, an important step to avail high-quality fishery products for consumers of the global market. There have been few studies that look into the influences of freeze-thaw cycles on the quality of selected fish species. Despite being limited in number, existing studies mostly considered a high level of temperature fluctuation leading to complete freeze-thaw cycles. Although, the high level of temperature fluctuation that leads to complete thawing of fillet may frequently occur in the cold chain, it is also important to consider lower levels of temperature fluctuation that might or might not result in partial thawing. The influence of such low levels of temperature fluctuation on the quality of frozen fillets is little known.

The main focus of this study was to investigate the effect of temperature fluctuation on some quality aspects of the frozen fillet. Drip loss, pH and fractional area of muscle fibers were studied for Atlantic salmon (*Salmo salar*) fillets stored at four different levels of temperature fluctuation.

MATERIALS AND METHODS

Samples: Samples of commercially frozen Atlantic salmon (*Salmo salar*) fillets originated from the same batch were supplied by the Colruyt group. The samples were immediately transferred into a freezer and kept at -18°C for 24 h. Then all fillets were randomly distributed into four groups in which each containing twenty fillets.

Simulation of temperature fluctuation: The experiment was designed to simulate different levels of temperature fluctuation in the logistic chain of frozen Atlantic salmon. The experimental conditions tested under the scope of this study included four conditions that represent temperature fluctuation during loading, transportation, loading and transportation and no fluctuation (ideal) conditions. This will enable us to identify the effect of temperature abuse during each process (loading, transportation) in the logistic system.

Storage experiment was conducted in two different refrigerators where the temperature record was taken daily after setting to the required degree. The total period of the storage experiment was 18 days for all groups of samples. The first group of samples named by No Fluctuation (NF) represented the ideal storage condition. Samples in this group were kept at -18°C for the total storage period. Samples in the second group named by Low Fluctuation (LF) represented a bad loading condition and samples in this group were stored at 18°C for 3 h and then kept at -18°C until the end of the experiment. Samples in the third group: High Fluctuation (HF) represented a bad transportation condition and stored at -12°C for 8 h followed by -18°C for 16 hours every day till the end of the experiment period. The last group of samples is labeled as very high fluctuation (VF), representing a condition in which both the loading and transportation are bad. Fillets in this group were once stored at 18°C for 3 h followed by -12°C for 8 h and -18°C for 16 h, every day for 18 days.

Drip loss: Fourteen fillet samples were dedicated for drip loss determination of each group following the respective storage experiment. To determine the drip loss, each sample was weighed before and after the storage experiment. At the end of the experiment period of eighteen days, all the samples were thawed overnight at 4°C and drained by tissue paper to remove excess moisture on the surface of the fillets. Finally, the samples were re-weighed and drip loss was expressed as a percentage of exudate:

$$\text{Drip loss(\%)} = \frac{(\text{Initial weight} - \text{Final weight})}{(\text{Initial weight})} \times 100$$

pH: Fourteen fillet samples from the four groups were thawed overnight before pH analysis. pH was measured using a benchtop pH meter (Hanna Instruments, Germany) by directly inserting the electrode into the middle of the fillet following a procedure described by Thorarinsdottir *et al.*^[9]. All measurements were performed at two different locations and the average result was reported.

Microscopic imaging: To analyze the microstructural changes of the fish muscle, six samples were taken from which two sub-samples were prepared from each. The sub-samples were placed in a small plastic container and containing Optimum Cutting Temperature (OCT) compound (embedding medium Tissue Tek USA) for sectioning. The prepared sub-samples were sectioned at 20 µm thickness using a microtome-cryostat (Microm HM 560 Cryostat, Thermo Fisher Scientific, Waltham, MA, USA) in an OCT environment where the temperature of the medium was maintained at -27°C. Microscopic images were taken using an inverted light microscope (VWR international, USA) fitted with Visicam 5.0 digital camera (VWR, Belgium).

Data analysis: Microscopic image analysis was performed using Image J software (National Institutes of

Health, Bethesda, Maryland, USA). One-way analysis of variance (1-way ANOVA) in combination with Tukey-Kramer HSD was carried out using JMP version 14.0.0; SAS Institute; Cary, NC for all mean comparison tests.

RESULTS AND DISCUSSION

Mean and standard deviation of percentage drip loss, pH and percentage fractional area of the space between muscle fibers are given in Table 1. Statistically significant differences were indicated using different letters while similar letters were used to indicate insignificant differences ($p>0.05$) with the highest value being labeled first.

Drip loss: Fillets stored at high-temperature fluctuation (HF) and fillets stored at very high-temperature fluctuation (VF) showed a higher drip loss compared to fillets stored at low-temperature fluctuation (LF) and those kept at constant frozen condition (NF). However, there was no difference between fillets stored at NF and LF conditions. Similarly, the difference between fillets stored at HF and VF was not significant.

The onset of melting temperature of Atlantic salmon, i.e., $T_m' = -17.1^\circ\text{C}$ is found between the fluctuating temperature region used in this study (-18°C , -12°C). This could have allowed ice crystals to melt and re-freeze in a process called re-crystallization^[7]. This temperature fluctuation might also have caused damage to muscle structure. Earlier studies reported significant damage to the muscle structure of Atlantic salmon due to temperature fluctuation^[10, 7]. Physical damage of muscle structures leads to relocation of intracellular water which, after thawing, remains permanently extracellular^[11]. The extracellular water measured as a drip loss showed higher in fillets stored at higher temperature fluctuation conditions (HF and VF) than in fillets stored at lower temperature fluctuation (NF and LF). The overall increasing trend in drip loss for fillets stored at high-temperature fluctuation is therefore attributed to the distractive effect of temperature fluctuation that led to damage of muscle structure. In agreement with this finding, Gang *et al.*^[12] reported higher drip loss in fillets stored at higher temperature fluctuation.

pH: Despite a slight difference in average pH values among all the groups, the variation between each group was insignificant ($p>0.05$). The slight reduction in pH of fillets stored at higher temperature fluctuation might indicate high electrolyte concentration which might be caused due to the high mobility of water in the fillets. A positive correlation between temperature fluctuation and water mobility in frozen Atlantic salmon was reported by Syamaladevi *et al.*^[7]. This finding is in agreement with a study by Fernandez-Segovia *et al.*^[13].

The fractional area between muscle fibers (%):

Figure 1 shows a microscopic image illustrating fish muscle structure. The space between muscle fibers is indicated using a grey color while the muscle fibers are colored in black. As illustrated in the figure, fillets in the NF group showed the smallest space between the muscle fibers followed by fillets in the LF group.

On the contrary, fillets in VF and HF groups showed a big gap between the muscle fibers. Statistical comparison of the area fraction (%) of the space between muscle fibers also confirmed the observed difference (Table 1). From the table, it is shown that the control group (NF) showed the smallest area fraction ($26.5\pm 8.77\%$) followed by LF ($37.31\pm 6.47\%$), HF ($50.37\pm 4.55\%$) and VF ($52.12\pm 2.81\%$). The smallest gap between muscle fibers in NF samples indicates the effect of temperature fluctuation was minimal compared to the other groups. LF group showed a higher gap between the muscle fibers and the damage on the muscle fibers is more pronounced as compared to the control group (NF).

In Fig. 1, a large difference was demonstrated in the HF group compared to the two former groups. The space between fibers was significantly higher and the damage on the muscle fibers was high. The last group (VF) which followed the whole storage period under fluctuating temperature showed the highest damage on the muscle fibers and a total disruption of the structure was observed.

The high gap between muscle fibers in LF, HF and VF groups might have been caused by bigger ice crystals formed during temperature fluctuation. Big ice crystals are associated with increased shrinkage and damage of muscle fibers in frozen fillets^[14].

Table 1: Mean and standard deviation of drip loss (%), pH and fractional area of the space between muscle fibers as influenced by temperature fluctuation

Groups	Drip loss (%)	pH	Area fraction (%)
-	Average±SD	Average±SD	Average±SD
NF	2.85±0.427 ^b	6.12±0.049 ^a	26.55±8.769 ^c
LF	2.86±0.424 ^b	6.10±0.059 ^a	37.31±6.473 ^b
HF	3.62±0.507 ^a	6.08±0.071 ^{ab}	50.37±4.555 ^a
VF	3.81±1.023 ^a	6.04±0.057 ^b	52.12±2.814 ^a

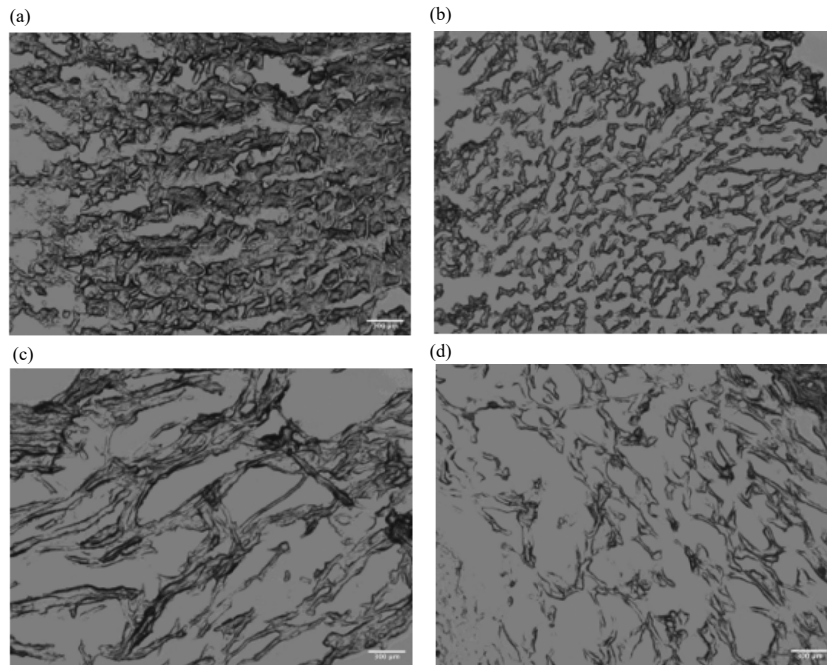


Fig. 1(a-d): Muscle microstructure of frozen fillets as affected by different levels of temperature fluctuation; (a), NF, (b), LF, (c) HF and (d), VF

CONCLUSION

In general, the quality of frozen Atlantic salmon fillets was negatively correlated with the level of temperature fluctuation. Drip loss and area fraction of the space between muscle fibers demonstrated a negative correlation while pH of fillets showed a positive correlation with temperature fluctuation. The relation between the obtained results from the three quality indicators confirmed the reduction of quality as a result of higher temperature fluctuation. Even though the increasing loss in quality was observed in the order of NF, LF, HF and VF groups, the effect of temperature fluctuation was more intense in HF and VF fillets. This can be observed from drip loss, pH and Area fraction, where all the parameters demonstrated high variation between the (NF, LF) and (HF, VF) fillets. The variation between the two (NF, LF) and (HF, VF) was statistically significant in drip loss and area fraction. In section 2.2, it is indicated that fillets in the NF group were stored at constant temperature and fillets in the LF group were exposed for short time temperature shock to represent fluctuation during a loading operation. Compared to fillets in HF and VF, where temperature fluctuation was very high, NF and LF fillets were stored at a more stable temperature. This was reflected in the results from quality parameters in which both NF and LF demonstrated only slight differences compared to the variation between the two groups from HF and VF fillets.

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