

## Research Article

# The Effect of Frozen Storage on the Quality of Atlantic Salmon

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### Abstract

Salmon is a nutritious fish food high in omega-3 fatty acids and is highly sought for its unique sensory attributes. Long term frozen storage allows the widespread distribution of salmon to improve the economic situation of this industry and avail a greater population to the nutritional benefits of salmon. The purpose of this research was to determine the quality changes in Atlantic Salmon stored at different freezer temperatures over 12 months. Fresh and pre-frozen salmon were placed in five different freezers set at -7°C, -12°C, -18°C, -29°C and -77°C and evaluated for quality at 90, 180, 270 and 360 days of storage. In general, quality was retained to a greater extent in salmon held at -29°C and -77°C for 360 days compared to other storage temperatures. No significant difference between freezer -29°C and -77°C was found in weight loss at days 180, 270 and 360, pore size at days 270 and 360, and for water

holding capacity, texture and TBARS, at days 90, 180, 270 and 360. The predicted shelf life of freshly frozen Atlantic Salmon was calculated based on the zero-order reaction model which was 268.82 days-7°C; 297.61 days-12°C; 355.78 days-18°C; 438.02 days-29°C, and 424 days-77°C. While the shelf life of pre-frozen Atlantic Salmon was 271.28 days-7°C; 281.77 days-12°C; 351.33 days-18°C; 392.59 days-29°C; and 402.99 days-77°C. Based on several quality parameters having minimal variation between 4 to 9 months at home freezer temperatures, energy savings could be realized by use of higher freezer temperatures for storage of salmon.

**Keywords:** Fresh and pre-frozen salmon; Freezing, thawing; Texture; Water holding capacity

## 1. Introduction

International fish intake has increased at a rate of 3.6% per year over the last 20 years partially due to the perceived health benefits of adding fish to the daily diet [1]. Many researchers have indicated that Atlantic salmon (*Salmo Salar*) shows cardiovascular, cancer inhibiting, and joint health benefits [2]. Atlantic salmon has omega-3 long chain fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), some of which are essential and important nutrients for human body. It is also a highly oily fish known to be low in mercury, like tuna, catfish, and cod [2]. So, the quality of Atlantic salmon is important for palatability during extended frozen storage [3]. While freezing will slow the biological, chemical, and physical deterioration of food, degradation of food quality such as color, texture, enzymatic activity, lipid oxidation, and ice crystal structural damage will still occur in the frozen state. Many researchers have reported that fast freezing results in rapid ice nucleation within the intracellular areas of food products. These ice crystals are smaller and more uniform, therefore cause less structural damage to the product [4]. The denaturation of the protein is one of the major problems caused by slow freezing with freezing temperature being a major factor impacting freezing rate [5]. Freezing at low temperatures resulted in small ice crystals and increased light scattering and absorption across all wavelengths in the visible region [6]. Several researchers showed that freezing and short-term storage changed the physical properties such as weight loss, color, and texture of Atlantic salmon and other types of fish [7]. Long term frozen storage leads to slow deterioration in the quality of salmon which can differ due to the storage temperature. Chemical reactions such as enzymatic activity and lipid oxidation are very important factors

affecting fish quality [8,9]. Lipid oxidation decreases the sensory quality of fish and fish products and is influenced by handling and processing of fish which can also impact nutritional quality, texture, and color [10,11]. The purpose of this research was to determine the effect of different holding temperatures on the quality of fresh and pre-frozen Atlantic salmon during long term storage.

## 2. Materials and Methods

### 2.1 Sample preparation

Fresh Atlantic salmon (*Salmo Salar*) fillets were purchased from a local fresh market, wrapped in sealed plastic bags, and stored in ice overnight. The next morning, fillets were sliced into ~60 to 65g samples (length = 7.3 cm, diameter = 4 cm, thickness = 2 cm), packaged under vacuum using plastic low-density polyethylene (LDPE) bags and stored in the refrigerator (3 hours) while preparing all samples. Half of the samples were pre-frozen samples at  $-77^{\circ}\text{C}$  (ultra-freezer) to a core temperature of  $-20^{\circ}\text{C}$  (~32 minutes). Then, fresh, and pre-frozen salmon samples were randomly placed into freezers at different freezing temperatures ( $-7^{\circ}\text{C}$ ), ( $-12^{\circ}\text{C}$ ), ( $-18^{\circ}\text{C}$ ), ( $-29^{\circ}\text{C}$ ) and ( $-77^{\circ}\text{C}$ ) for 360 days (Figure 1). Each freezer was connected to a sensor to measure internal freezer temperature and the internal freezer relative humidity. One sample in each freezer was connected to a sensor to measure the core fillet temperature. The quality attributes tests for the fresh and pre-frozen salmon were conducted on days 0, 30, 90, 180, 270 and 360. Day 0 sampling was taken for the pre-frozen samples after being placed at  $-77^{\circ}\text{C}$  (ultra-freezer) and reaching a core temperature of  $-20^{\circ}\text{C}$  (~32 minutes) while the fresh samples were removed from each freezer at each endpoint temperatures ( $-7^{\circ}\text{C}$ ), ( $-12^{\circ}\text{C}$ ), ( $-18^{\circ}\text{C}$ ), ( $-29^{\circ}\text{C}$ ) and ( $-77^{\circ}\text{C}$ ) for testing. On day 0, the samples stored at  $7^{\circ}\text{C}$ ,  $12^{\circ}\text{C}$ ,  $18^{\circ}\text{C}$ ,  $-29^{\circ}\text{C}$

and -77°C freezers were removed for analysis after 861, 420.5, 309.5, 310.5, 226.5 minutes, respectively (Table 1). After freezing, one sample of fresh and pre-frozen was freeze-dried to conduct the scanning electron microscopy (SEM) to determine the surface

pore numbers and size. Freeze loss and lightness (L\*) were measured before and after thawing. Other tests were conducted after thawing in the refrigerator (3.33°C) (38°F) for 24 hours.

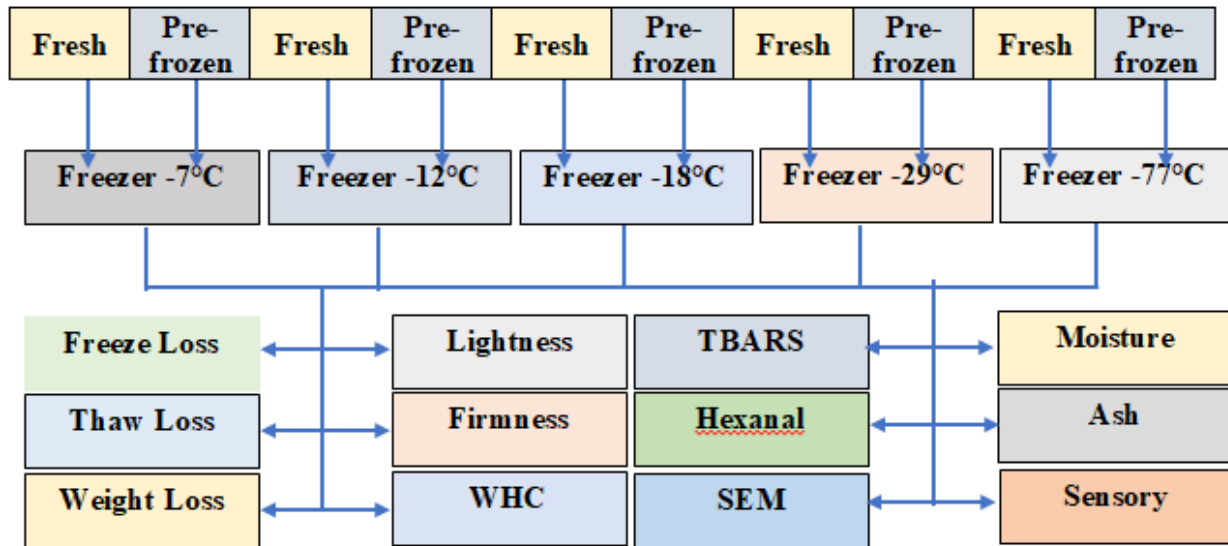


Figure 1: Experimental flowchart

No	Freezer Temperature	Average Freezing Time (Minutes)	Average Freezing Rate (°C Minute)
1	7°C (Core temperature is 7°C)	861.0±28.28	0.025±0.007
2	12°C (Core temperature is 12°C)	420.5±40.31	0.075±0.007
3	18°C (Core temperature is 18°C)	309.5±0.71	0.140±0.000
4	-29°C (Core temperature is -29°C)	310.5±21.92	0.205±0.007
5	-77°C (Core temperature is -77°C)	226.5±2.12	0.640±0.000

Table 1: Freezing times for treatments frozen at different freezing rates (freezer temperature environment) to the same core temperatures

### 2.2 Freeze loss

The fresh and pre-frozen samples were weighed before each salmon sample was placed into their respective freezers and then again after freezing on a Mettler Toledo PB3002 scale (Langacher Greifensee, Switzerland). The percent freeze loss was calculated

based on this equation:

$$\% \text{ Freeze loss} = \frac{(\text{Weight before freezing} - \text{weight after freezing})}{\text{Weight before freezing}} * 100$$

### 2.3 Thaw loss

The fresh and pre-frozen salmon samples were

weighed after freezing and then again after thawing on a Mettler Toledo PB3002 scale (Langacher Greifensee, Switzerland). The percent thaw loss was calculated based on this equation:

$$\% \text{ Thaw loss} = [(\text{Weight after freezing} - \text{weight after thawing}) / \text{Weight after freezing}] * 100$$

#### 2.4 Weight loss

A total percent weight loss was calculated by summing the percent freeze loss and percent thaw loss. The percent weight loss was calculated based on this equation:

$$\% \text{ Weight loss} = (\% \text{ Freeze loss} + \% \text{ Thaw loss})$$

#### 2.5 Lightness

Lightness (L\*) was measured on fresh and frozen salmon samples using a Minolta Colorimeter with a DP-400 data processor and CM-400 Chroma Meter (Minolta, Colorado). The lightness (L\*) for frozen salmon was measured immediately after weighing of the sample bag. The influence of the bag on color was accounted for during calibration. The lightness was measured on samples after freezing and after thawing.

#### 2.6 Texture (firmness)

Texture analysis was conducted after thawing using a TA XT plus TA-90 Texture Analyzer interfaced with Exponent Stable Microsystems Version 6,1,1,0 software (Scarsdale, New York). A Muellenet-Owens's razor shear blade method was used with a test speed of 5 mm/sec, return speed of 10 mm/sec, target mode of distance, distance of 15 mm, trigger type of auto (force), and a trigger force of 10 g. The instrument was calibrated with a 2000 g weight calibration.

#### 2.7 Water Holding Capacity (WHC)

As described by [12], Whatman® #3 filter paper (Sigma-Aldrich, USA) was folded in a tube shape inside the centrifuge tubes to absorb excessive moisture released during centrifugation. Approximately 10 g of minced muscle was weighed accurately and immediately centrifuged (Eppendorf 5804R, Germany) at 269 x g (1500 rpm) for 15 min at 4°C. After centrifugation, the samples were reweighed, excluding the moisture absorbed by filter paper. The weight loss after centrifugation was divided by the initial weight and expressed as % WHC. % WHC = [(Initial weight (g) – the moisture absorbed by filter paper (g) / initial weight)] x 100. The moisture absorbed by filter paper = filter weight after centrifuge – filter weight before centrifuge.

#### 2.8 Determination of Thiobarbituric Acid Reactive Substances (TBARS)

TBARS was determined in duplicate for each replication as described by [13] with modifications. Five g of wet salmon sample was placed in a 45 ml conical-bottom disposable plastic tube (Corning™, VWR North American Cat. No 21008–105, USA). 10 ml deionized water was added to the same tube containing salmon and homogenized by hand-held homogenizer for 20 sec. One ml was taken from the 45-ml tube and added to 15 ml test tube. Three ml of TBA/TCA solution (0.7208 g of TBA dissolved in boiling water + 15 g of TCA dissolved then made up to 250 ml with deionized water). The sample was mixed using a vortex for 1 minute and then heated at 90°C for 15 minutes. The sample was cooled and mixed by vortex for 1 minute. The sample was then centrifuged (Eppendorf 5804R, Germany) at 14000 x g for 10 minutes using. The supernatant was filtered using 0.45 µm GHP Membrane Acrodisc (Pall Corporation, USA) and 2 ml of the filtered liquid was placed in another tube with 4 ml TBA solution. The

sample was added to a 96-well microplate (0.25 ml) to measure the absorbance (Spectronic® 20 GENESYSTEM™, USA) at 532 nm. TBARS values were obtained from a standard curve prepared using concentrations from 0 µL to 70 µL of a mixture of 1,1,3,3-tetrathoxypropane solution (TEP) and H<sub>2</sub>O solutions with 4 ml of TBA solution. The tubes used for the standard curve were placed in a 90°C water bath and measured the absorbance at 532 nm.

## 2.9 Volatile headspace analysis (Gas Chromatography)

Salmon samples (7.5 g) were sealed with Teflon septa and aluminum caps in 15 ml GC vials then heated at 90°C for 15 minutes in a headspace auto sampler (HP7694 Hewlett Packard, Wilmington, DE). The vial headspace was automatically injected onto the head of HP5-MS 95% dimethyl-siloxane copolymer capillary column (30 mx 250 µm x 0.25 µm) (Agilent Technologies, Inc., CA, USA) with a flow rate of 1.5 mL/min and integrated with a gas chromatograph (HP6890 GC-MS system, Hewlett Packard, Wilmington, DE). Peak areas were recorded for hexanal and compared between treatments.

## 2.10 Ice Crystal Pore Analysis

One sample from fresh and pre-frozen salmon (per replication) were freeze-dried (Labconco Lyph-lock 6 Freeze Dry System, Kansas City, Missouri) for five days to remove moisture and stabilize ice crystal pores. Freeze drying was achieved under a condenser temperature range of -44°C to -48°C and a vacuum pressure range of  $50 \times 10^{-3}$  to  $500 \times 10^{-3}$  millibars. Fresh and pre-frozen samples were removed from the freeze dryer after five days and stored in a 3.33°C (38°F) refrigerator overnight before microscopy analysis. The surface and core areas of the freeze-dried treatments was analyzed with a S-3400N

Variable-Pressure SEM (VP-SEM) (Hitachi High-Technologies Corporation, Clarksburg, Maryland). The microscope was used to capture micrograph images of pores that are comparable to ice crystals formed within the tissue during freezing. Samples were first subjected to surface ice crystal damage analysis and then sliced in half to observe core ice crystal damage analysis. The instrument was set to a BSE detector setting with an accelerating voltage of 20 kV and a chamber pressure of 40 Pa.

## 2.11 ImageJ Ice Crystal Pore Size Analysis

Image J 1.50i was created by Wayne Rasband and the National Institutes of Health. This software was used to quantify ice crystal pore damage of the micrograph images obtained from the S-3400N Variable-Pressure Scanning Electron Microscope. Each image was set to a scale of known distance of 500 µm, distance in pixels of 500, and a pixel aspect ratio of 1.0. Data gathered was included area, area fraction, and a fit ellipse, each to a decimal place of 3. Each pore was analyzed based upon pore size from 50 µm<sup>2</sup> to infinity and circularity from 0 to 1.0. Based upon these settings, the software produced a total pore count, total pore area, average pore size, percent area, major and minor axes values, and pore angle data.

## 2.12 Moisture Content Determination

An empty dish and lid were dried in the oven at 105°C for 3 hours and transferred to desiccator to cool. The empty dish and lid were weighted before adding samples. Three g of samples was weighed into a dish then uniformly spread and placed in an oven (105°C) over night. The dish was cooled and weighted again after drying. The moisture content was calculated based on the following equation:

$$[(\text{weight 1} - \text{weight 2}) / \text{weight 1}] * 100$$
 Where weight 1 = sample weight before drying, weight 2 =

sample weight after drying.

### 2.13 Ash Content Determination

The ash content of salmon was determined using Official AOAC methods [14]. Briefly, 2 to 3 g of sample was placed in a dried crucible then held in a muffle furnace at 525°C for 20 hours until white ash is obtained, then % ash was determined gravimetrically.

### 2.14 Sensory Evaluation

The Quality Index Method (QIM) scheme was applied for the sensory evaluation of salmon (flesh) fillets (*Salmo salar*) [15] with some modifications. Eight subjects experienced in sensory evaluation of fish evaluated the salmon (flesh) fillet samples. The sensory evaluation was performed using a 12-point scale [15] (1 being the highest quality score and 12 the lowest). Five ~25g pre-frozen and five fresh-frozen salmon samples (after thawing) were placed randomly on a clean table 15 min before the evaluation. The samples were at room temperature and under white fluorescent light. The (flesh) fillet was evaluated for:

- a. color (0 = Normal salmon color, 1 = slightly grey hue, 2 = Grey hue, yellowish near the abdomen).
- b. brightness (0 = Shiny, 1 = slightly mat, 2 = dull).
- c. odor (0 = Neutral, cucumber, 1 = Melon, 2 = Slight sour, slightly overripe fruit, 3 = Blue cheese, overripe fruit, spoilage sour);
- d. texture (0 = Very firm, 1 = Less firm, 2 = Soft); and
- e. gaping (0 = Gaping, less than 10%, 1 = Gaping, 10-20%, 2 = Gaping, 25-50%, 3 = Gaping more than 50%). Gaping is when the flakes that are originally connected to each other by connective tissues separate, and the fillet loses appearance of a continuous muscle [16]. Gaping is negative attribute

of the appearance of fillets, making them difficult to sell and makes skinning difficult. Gaping is caused by rupture of the connective tissue, which produces flaking of the fillet. The cause of gaping can crudely be described as the interaction between forces pulling the muscle apart, and the strength of the tissue [16]. A total QIM score of 8 out of 12 is the quality limit was considered a poor-quality sample.

### 2.16 Shelf-Life Calculation - Zero Order Reaction Model

Shelf-life estimation was based on the sensory Quality Index Scale with the score of 8 as the limit for acceptability. The correlation between storage period and sensory evaluation was analyzed using a linear regression equation. The sensory responses were plotted against time for each storage temperature. The regression equation and the slope value for each plot has been used to for Zero order reaction model to predict the shelf life of salmon.

$$\text{Shelf-Life} = (A_e - A_0) / K$$

$A_e$  is the quality limit,  $A_0$  is the quality indicator value in day 0,  $K$  is the slope (Reaction Rate Constant).

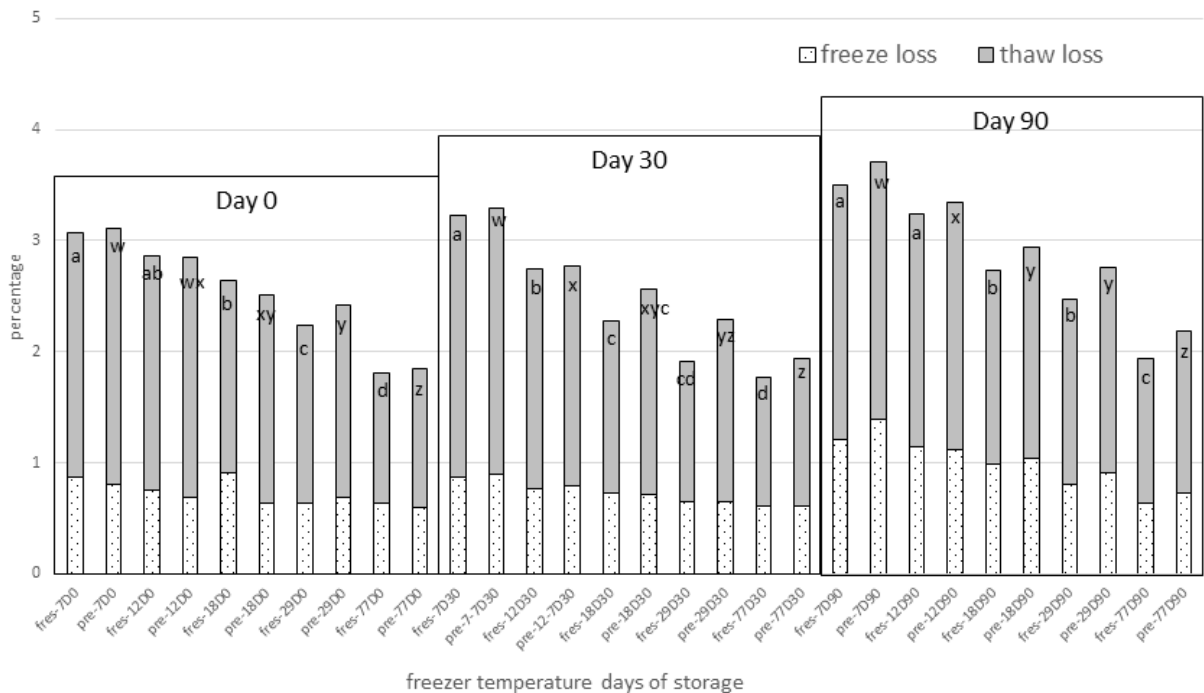
### 2.17 Statistical analysis

All treatments were randomly assigned to the salmon samples using a completely randomized design. The experiment was replicated 3 times on different days using different lots of salmon. The data were analyzed using a one-way analysis of variance (ANOVA) and statistical significance was at the 5% level. For analyses where freezing had significant treatment effect ( $P \leq 0.05$ ) significant differences were determined using multiple comparison tests; least significant difference (LSD) and Tukey's test for significance.

### 3. Results and Discussion

Weight loss for salmon increased for all storage temperatures over the 1- year storage study ( $P \leq 0.05$ ) (Figure 2 and 3). Salmon samples stored at  $-7^\circ\text{C}$  had a significantly higher weight loss than other temperatures while salmon samples held at  $-77^\circ\text{C}$  had a lower weight loss on each sampling day. No significant difference between pre-frozen and fresh salmon at all temperatures on days 30 and 90. Poovarodom et al. [17] observed that the weight loss increased rapidly with storage time (6% after a 9-month storage period at  $-20^\circ\text{C}$ . Campañone et al. [18]

monitored weight loss of meat during freezing and frozen storage and found a range of 0.28%-2.98% during the freezing process. Weight loss within frozen and thawed salmon occurred because of damaged induced by ice crystal growth during the freezing process [19]. Salmon frozen at higher temperatures freeze more slowly and as a result accrue larger, less uniform ice crystals. As ice crystals form in extracellular and intracellular areas around fish muscle structure, cell membrane damage causes less water to be bound within the muscle structure.

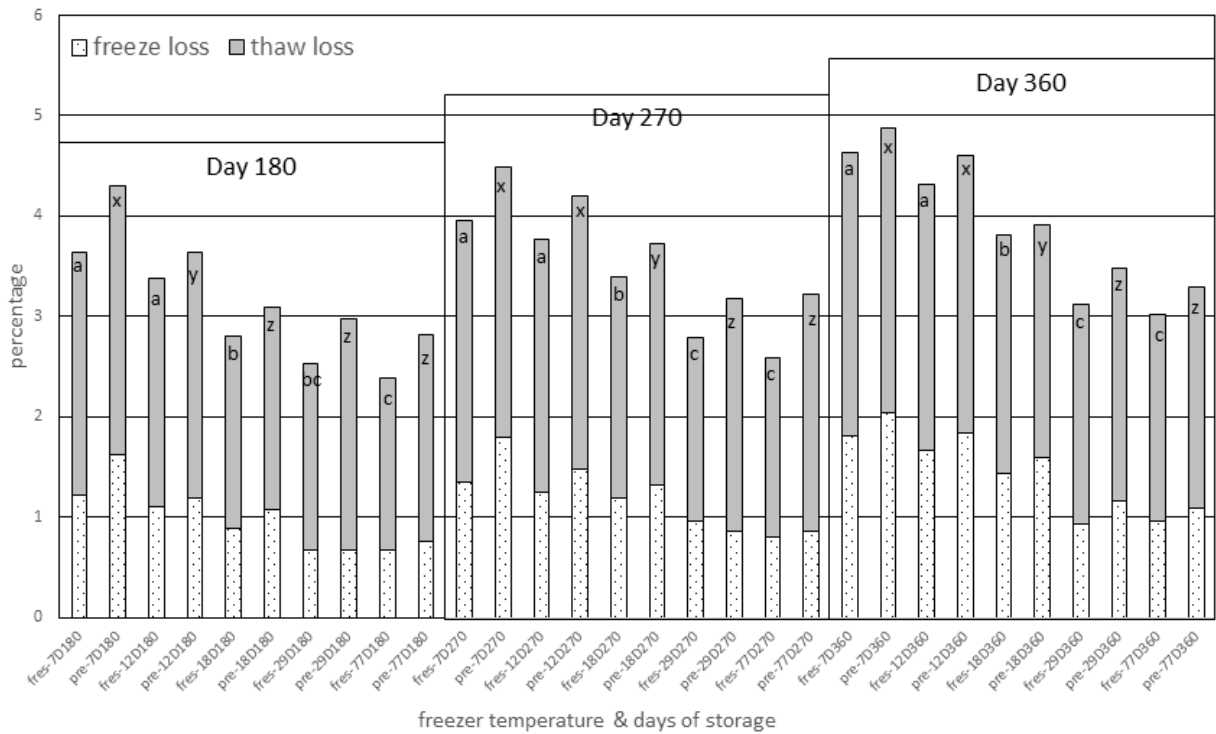


**Figure 2:** Weight loss during frozen storage, thawing and combined weight loss for fresh and pre-frozen salmon stored at different temperatures for 0, 30 and 90 days. n=3. fres = fresh peaches; pre = prefrozen peaches.

<sup>a-d</sup> means for fresh samples within each day of storage with different superscript are significantly different ( $P \leq 0.05$ ).

<sup>w-z</sup> means for pre-frozen samples within each day of storage with different superscript are significantly different ( $P \leq 0.05$ ).





**Figure 3:** Weight loss during frozen, thawing, and combined weight loss for fresh and pre-frozen salmon stored at different temperatures for 0, 30 and 90 days. n=3. fres = fresh peaches; pre = prefrozen peaches.

<sup>a-c</sup> means for fresh samples within each day of storage with different superscript are significantly different (P≤0.05).

<sup>x-z</sup> means for pre-frozen samples within each day of storage with different superscript are significantly different (P≤0.05).

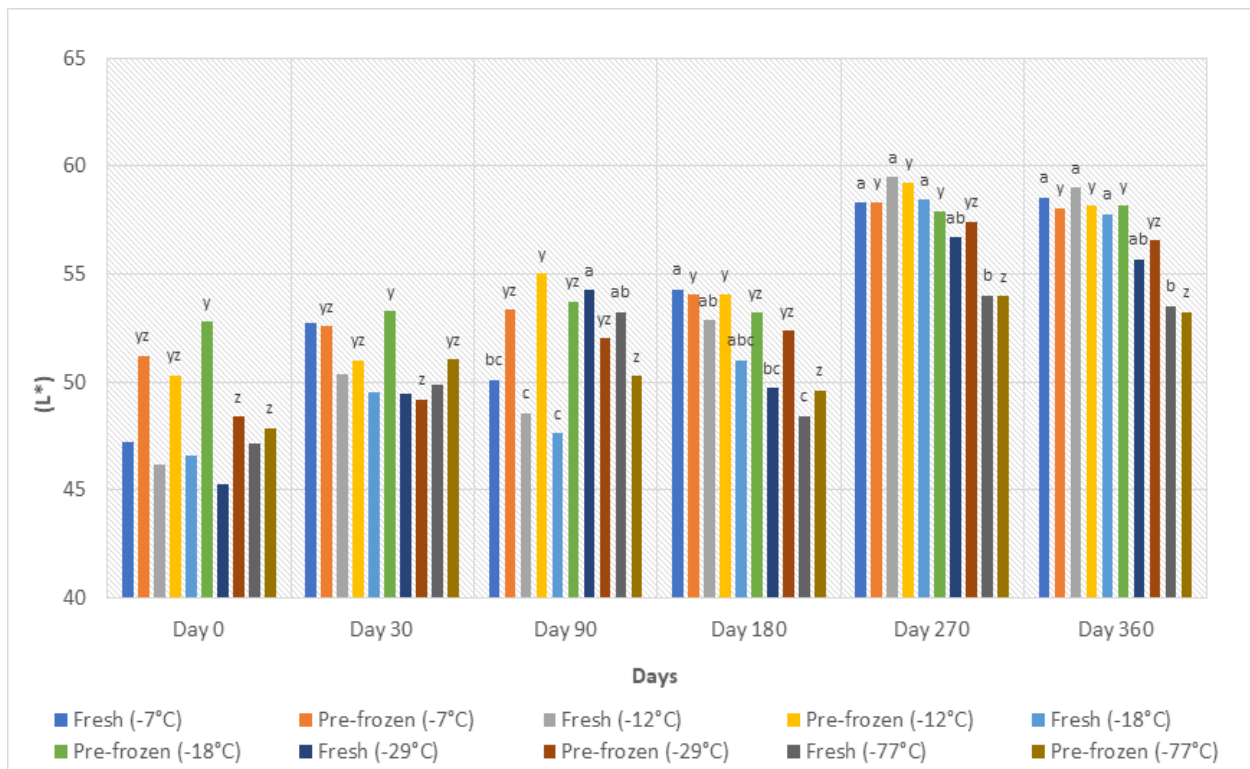
Thawing can further damage meat structure and the period during which the damage from slow freezing manifests itself. During thawing ice crystals melt, and if formed intracellularly or around muscle tissue, moisture would remain within the fish [20]. Salmon lightness (L\*) after thawing increased over 1-year storage (P≤0.05) (Figure 4). The fresh samples stored at -77°C had lower L values than other fresh samples stored at -18°C and -12°C and -7°C on days 180, 270 and 360 (P≤0.05). The pre-frozen samples stored at -77°C had lower L values than other pre-frozen samples stored at -12°C and -7°C on days 180, 270 and 360 (P≤0.05). Color can affect product perception without affecting nutrition or flavor [21]. Fading or increase in lightness is related to ice crystal

formation during freezing [6]. Higher freezing rates form small, more numerous ice crystals within salmon, which then reflect light more intensely. Slower freezing rates form larger and fewer ice crystals in salmon, resulting in light refraction and a darkening effect of the meat surface. Firmness of salmon fillets increased over time (P≤0.05) (Figure 5). The fresh and pre-frozen samples stored at -77°C was less firm than other fresh and pre-frozen samples stored at -7°C on day 360 (P≤0.05). Frozen storage temperature affected texture quality in Atlantic salmon fillets more than thawing techniques [19]. Texture changes during frozen storage have also been directly linked to protein denaturation within fish [22]. Water holding capacity (WHC) decreased for



all samples at all temperatures over the 1- year storage ( $P \leq 0.05$ ) (Figure 6). The fresh and pre-frozen samples stored at  $-29^{\circ}\text{C}$   $-77^{\circ}\text{C}$  had higher WHC than other fresh and pre-frozen samples stored at  $-7^{\circ}\text{C}$  and  $-12^{\circ}\text{C}$  on days 30, 90, 180, 270, and 360 ( $P \leq 0.05$ ). Water holding capacity is closely related to textural properties, and a low of WHC is often related to

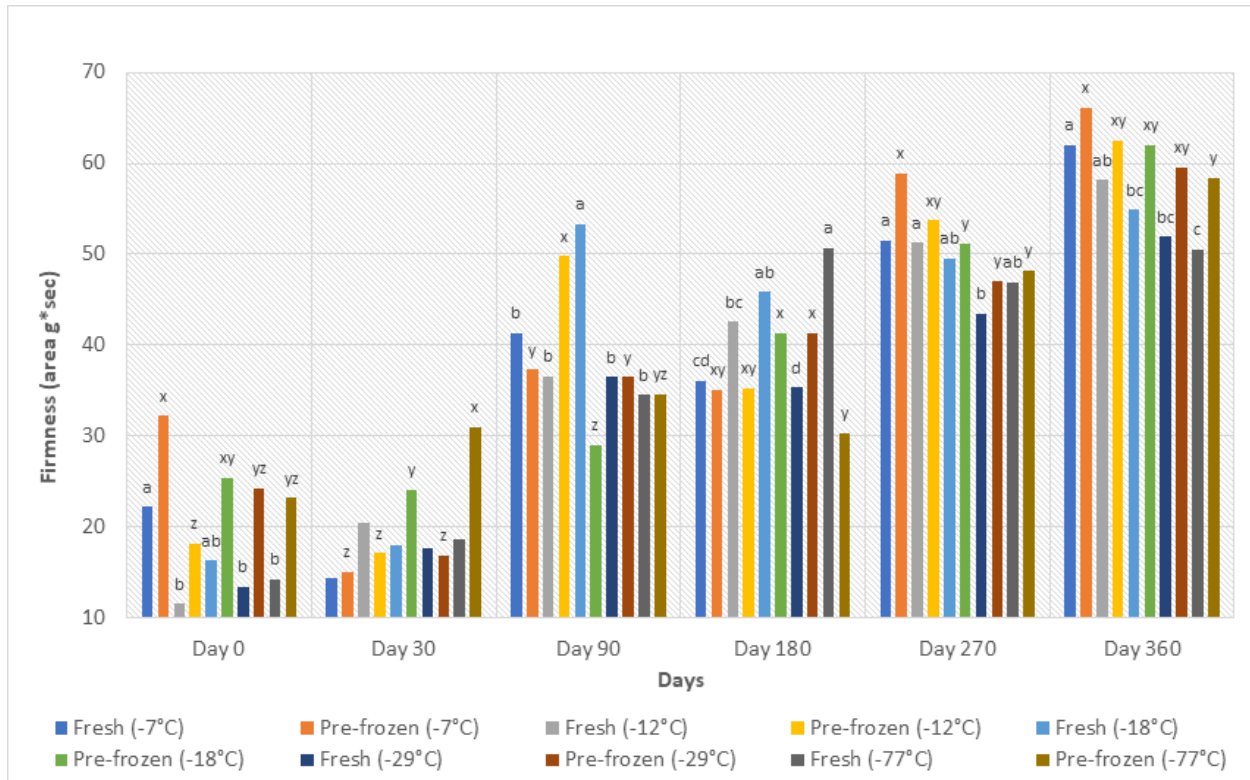
postmortem structural changes in the muscle. Changes include myofilament lattice degradation, denaturation of myosin, and increase of extracellular space [23]. The decrease in WHC may result from proteolytic activity in the muscle during storage [24] which causes a loss of water described as a “leaking out” effect [25].



**Figure 4:** Average lightness ( $L^*$ ) of fresh and pre-frozen salmon held at different temperatures (after thaw) for 1 year.  $n=9$ .

<sup>a-c</sup> means for fresh samples within each day of storage with different superscript are significantly different ( $P \leq 0.05$ ).

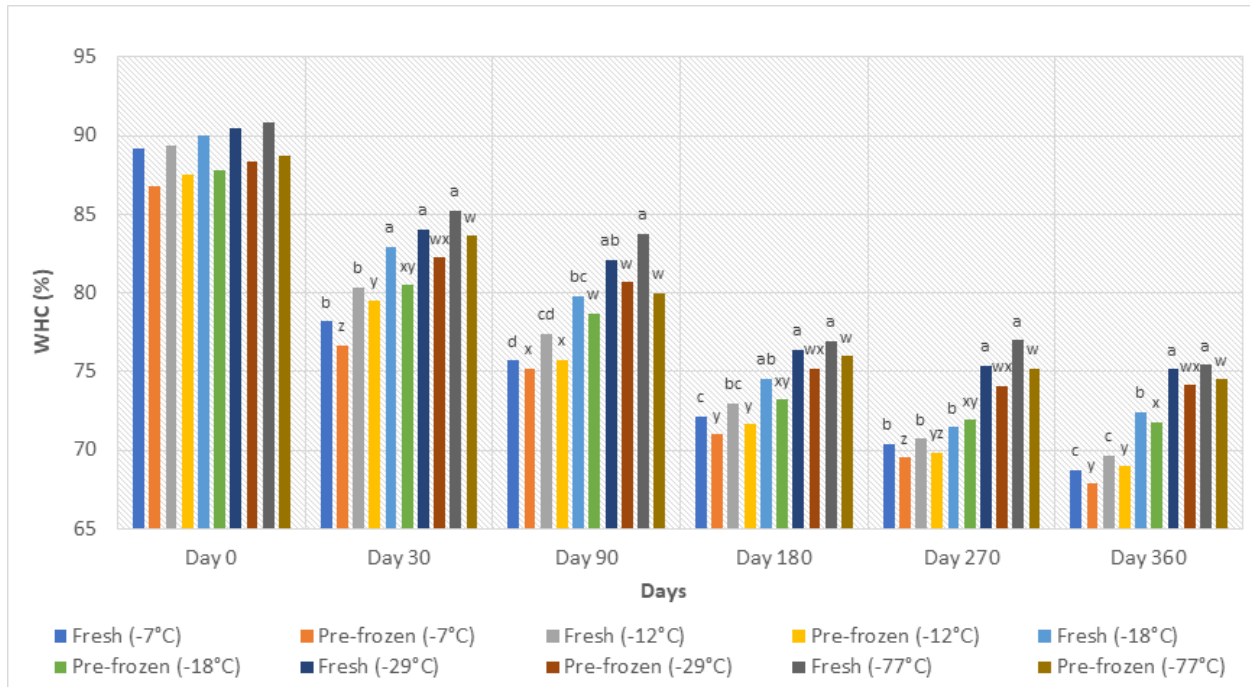
<sup>y-z</sup> means for pre-frozen samples within each day of storage with different superscript are significantly different ( $P \leq 0.05$ ).



**Figure 5:** Average firmness (area g\*sec) of fresh and pre-frozen salmon frozen at different temperatures and held for 1 year. n=9.

<sup>a-d</sup> means for fresh samples within each day of storage with different superscript are significantly different (P≤0.05).

<sup>x-z</sup> means for pre-frozen samples within each day of storage with different superscript are significantly different (P≤0.05).



**Figure 6:** Average water holding capacity (% WHC) of fresh and pre-frozen salmon frozen at different temperatures and held for 1 year. n=9.

<sup>a-d</sup> means for fresh samples within each day of storage with different superscript are significantly different ( $P \leq 0.05$ ).

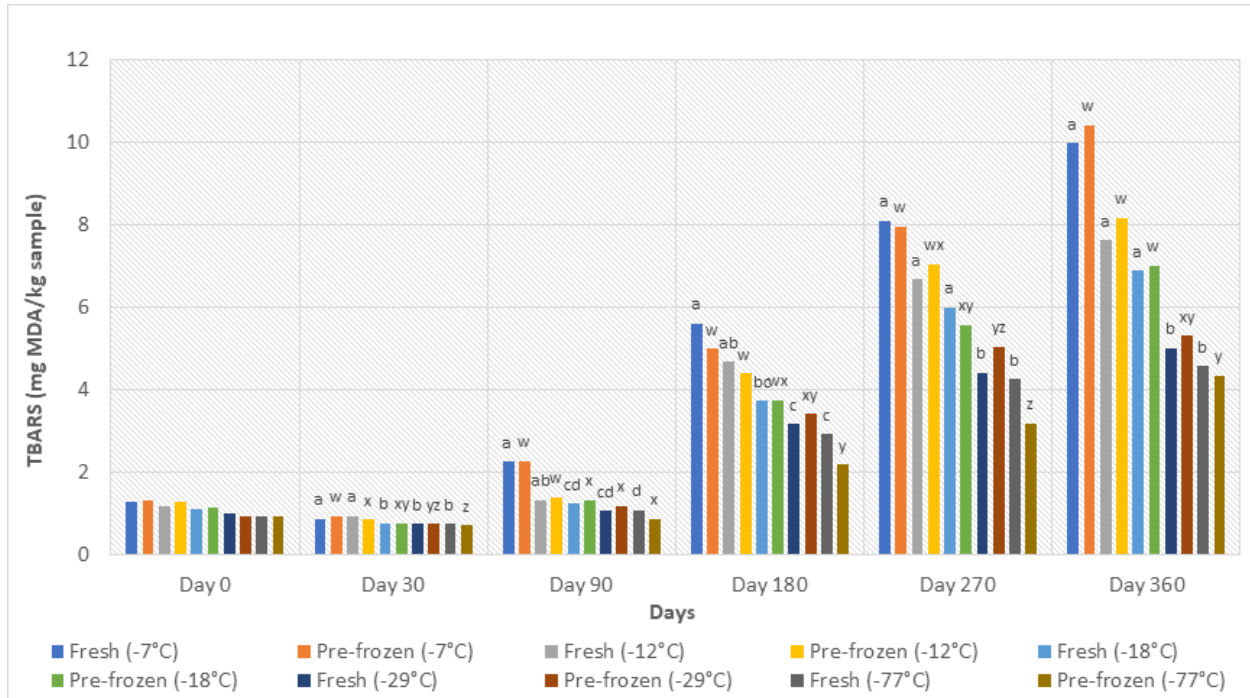
<sup>w-z</sup> means for pre-frozen samples within each day of storage with different superscript are significantly different ( $P \leq 0.05$ ).

The hexanal was detected in day 270 in all samples at all temperatures. However, the peak area of hexanal in all samples stored at  $-29^\circ\text{C}$  and  $-77^\circ\text{C}$  were lower than samples stored at  $-18^\circ\text{C}$ ,  $-12^\circ\text{C}$  and  $-7^\circ\text{C}$  ( $P \leq 0.05$ ). The TBARS increased over time ( $P \leq 0.05$ ) (Figure 7), but the fresh and pre-frozen samples stored at  $-29^\circ\text{C}$  -  $-77^\circ\text{C}$  had lower TBARS than other fresh and pre-frozen samples stored at  $-7^\circ\text{C}$  and  $-12^\circ\text{C}$  on days 30, 90, 180, 270, and 360 ( $P \leq 0.05$ ). The TBARS value primarily quantifies malondialdehyde which is an indicator of lipid oxidation and was found to increase in numerous studies during the storage of fish [26,27]. The number of pores decreased while the size of pores increased for salmon samples over time ( $P \leq 0.05$ )

(Figure 8 and Figure 9). Samples stored at  $-77^\circ\text{C}$  and  $-29^\circ\text{C}$  were had more pores than other temperatures on days 90, 180, 270 and 360 ( $P \leq 0.05$ ) while,  $-7^\circ\text{C}$  was lower in pore number than other temperatures ( $P \leq 0.05$ ). The largest pore sizes were detected in samples stored at  $-7^\circ\text{C}$  on days 30, 90, 180, 270 and 360 ( $P \leq 0.05$ ). Salmon samples stored at  $-29^\circ\text{C}$  and  $-77^\circ\text{C}$  had smaller pores than samples stored at  $-7^\circ\text{C}$  and  $-12^\circ\text{C}$  on days 30, 90, 180, 270 and 360 ( $P \leq 0.05$ ). Ice crystal damage may be attributed to the number of nucleated ice crystals first and then to the specific average size of the ice crystals formed [4]. Since the food surface freezes more quickly than the center, core ice crystal nucleation and morphology changes occur more slowly than those on the surface.

Studies have shown that the differences in ice crystal characteristics on the food surface reflect those seen in the center of food products due to freezing rate but

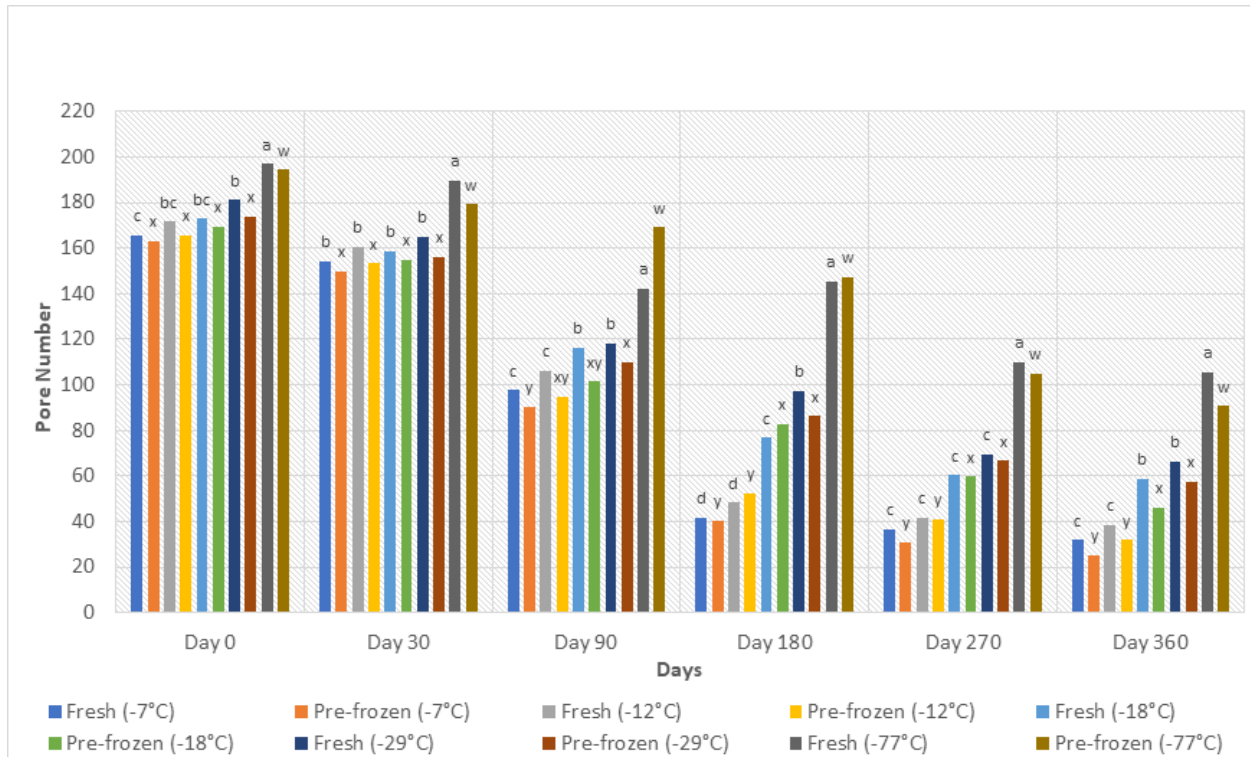
pore number and average size at the center of cylindrical gelatin gels decreased with increasing diameter when frozen at the same freezing rate [28].



**Figure 7:** Average TBARS (mg MDA/kg sample) of fresh and pre-frozen salmon frozen at different temperatures and held for 1 year. n=9.

<sup>a-d</sup> means for fresh samples within each day of storage with different superscript are significantly different ( $P \leq 0.05$ ).

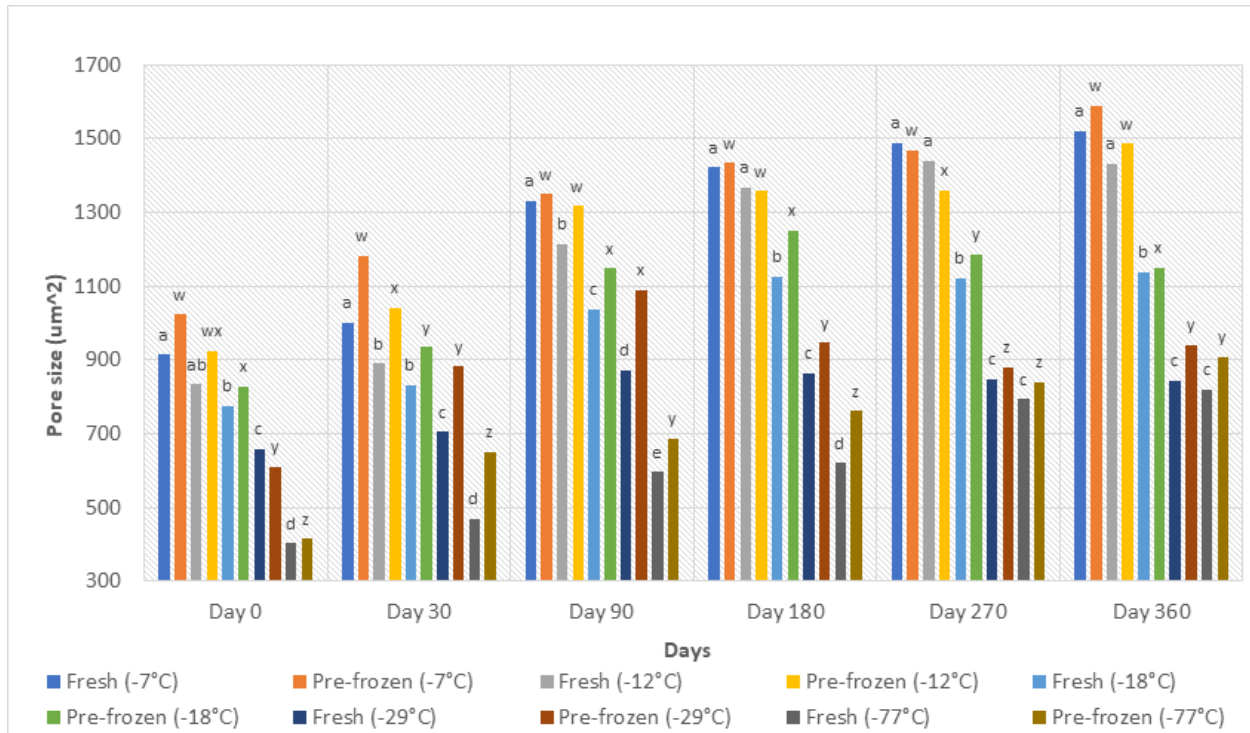
<sup>w-z</sup> means for pre-frozen samples within each day of storage with different superscript are significantly different ( $P \leq 0.05$ ).



**Figure 8:** Scanning electron microscopy (pore numbers) of fresh and pre-frozen salmon frozen and held for 1 year at different temperatures. n=9.

<sup>a-d</sup> means for fresh samples within each day of storage with different superscript are significantly different (P≤0.05).

<sup>w-z</sup> means for pre-frozen samples within each day of storage with different superscript are significantly different (P≤0.05).



**Figure 9:** Scanning electron microscopy (pore size  $\mu\text{m}^2$ ) (500  $\mu\text{m}$ ) of fresh and pre-frozen salmon frozen and held for one year at different temperatures.  $n=9$ .

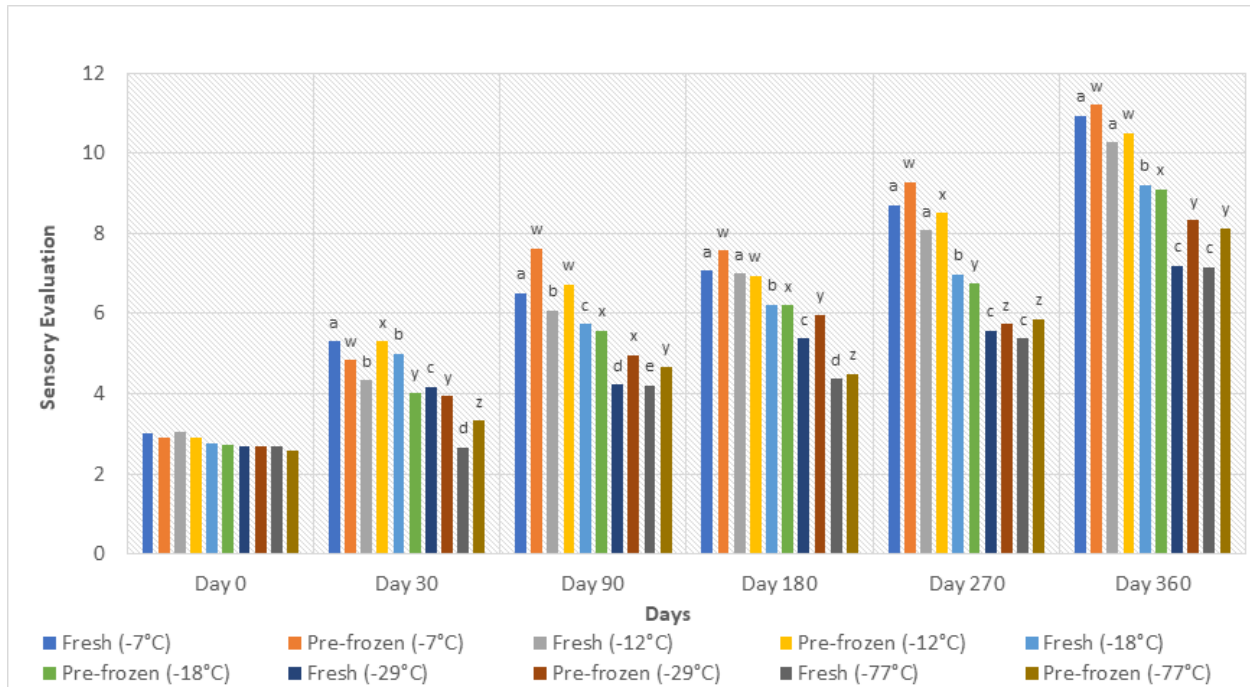
<sup>a-e</sup> means for fresh samples within each day of storage with different superscript are significantly different ( $P \leq 0.05$ ).

<sup>w-z</sup> means for pre-frozen samples within each day of storage with different superscript are significantly different ( $P \leq 0.05$ ).

The moisture content decreased during 1-year storage ( $P \leq 0.05$ ) while ash content increased ( $P \leq 0.05$ ). However, there was no difference in moisture content among all samples due to storage temperature for 1-year storage ( $P > 0.05$ ). The sensory evaluation score of salmon decreased during 1-year storage ( $P \leq 0.05$ ) (Figure 10). The fresh and pre-frozen samples stored at  $-29^\circ\text{C}$   $-77^\circ\text{C}$  were judged to be higher quality than other fresh and pre-frozen samples stored at  $-7^\circ\text{C}$ ,  $-12^\circ\text{C}$  and  $-18^\circ\text{C}$  on days 30, 90, 180, 270, and 360 ( $P \leq 0.05$ ). The QIM includes sensory attributes of odor, gaping and texture and the acceptable quality limit was 8 out of 12. The shelf life of salmon was

predicted according to the sensory evaluation quality limit. The estimated shelf life of freshly frozen Atlantic Salmon suggested 268.82 days at  $-7^\circ\text{C}$ ; 297.61 days at  $-12^\circ\text{C}$ ; 355.78 days at  $-18^\circ\text{C}$ ; 438.02 days at  $-29^\circ\text{C}$ , and 424.00 days at  $-77^\circ\text{C}$ . While the shelf life of pre-frozen Atlantic Salmon was 271.28 days at  $-7^\circ\text{C}$ ; 281.77 days at  $-12^\circ\text{C}$ ; 351.33 days at  $-18^\circ\text{C}$ ; 392.59 days at  $-29^\circ\text{C}$ ; and 402.99 days at  $-77^\circ\text{C}$  (Table 2). The correlation of sensory evaluation with TBARS and weight loss for all treatments were significantly correlated to the sensory evaluation ( $P \leq 0.05$ ).





**Figure 10:** Sensory evaluation of fresh and pre-frozen salmon frozen and held for one year at different temperatures. n=9. (Higher score = lower quality).

<sup>a-e</sup> means for fresh samples within each day of storage with different superscript are significantly different (P≤0.05).

<sup>w-z</sup> means for pre-frozen samples within each day of storage with different superscript are significantly different (P≤0.05).

Quality factor	Treatment	Regression equation (Zero order)	Correlation Coefficient (R2)	Reaction Rate Constant (K)	Shelf-Life (Days)
Sensory evaluation	Fresh at -77°C	y = 0.0125x + 2.5919	0.577	0.0125	424
	Pre-frozen at -77°C	y = 0.0134x + 2.7794	0.5822	0.0134	402.99
	Fresh at -29°C	y = 0.0121x + 3.0842	0.592	0.0121	438.02
	Pre-frozen at -29°C	y = 0.0135x + 3.0848	0.5927	0.0135	392.59
	Fresh at -18°C	y = 0.0147x + 3.6379	0.6564	0.0147	355.78
	Pre-frozen at -18°C	y = 0.015x + 3.4366	0.663	0.015	351.33
	Fresh at -12°C	y = 0.0167x + 3.9213	0.7916	0.0167	297.61
	Pre-frozen at -12°C	y = 0.0181x + 3.9616	0.7359	0.0181	281.77
	Fresh at -7°C	y = 0.0186x + 4.0506	0.7447	0.0186	268.82
	Pre-frozen at -7°C	y = 0.0188x + 4.4644	0.7696	0.0188	271.28

**Table 2:** Predicted shelf life of fresh and pre-frozen salmon



#### **4. Conclusion**

The predicted shelf life of fresh Atlantic Salmon based on sensory scores was - 7°C (268.81 days); - 12°C (297.60); -18°C (355.78); -29°C (438.01), and - 77°C (424.00). While the predicted shelf life of pre-frozen Atlantic Salmon was - 7°C (271.27 days); - 12°C (281.76); -18°C (351.33); -29°C (392.59), and - 77°C (402.98). Thus, freezing of Atlantic salmon even at the highest temperature gave 268.81 and 271.27 days shelf life for the fresh and pre-frozen salmon, respectively and this could reduce energy cost if this shelf life was sufficient for the consumer as compared to higher freezer temperatures. The fresh and pre-frozen samples of Atlantic salmon stored at -29°C and -77°C were significantly smaller in pore size than samples stored at -7°C and -12°C. Therefore, the temperature of -29°C would be better than -18°C, -12°C, and -7°C to maintain the quality of Atlantic salmon.

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#### **Conflict of interest**

The authors declare no conflict of interests.

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