Research Article

Post-mortem quality changes in rainbow trout (*Oncorhynchus mykiss*) during live storage

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Abstract

This study aimed to examine the quality changes of rainbow trout (*Oncorhynchus mykiss*) caught from the farm and live supply center at different times. Rigor mortis, ATP-related components, K-value, and blood parameters of rainbow trout were monitored during storage in the live supply center of rainbow trout. The progress of rigor mortis was accompanied by an increase in storage hours. The initial K-value of fish in live storage was lower than 8%. K-value of the fish climbed to more than 50% at 24 h. After 28 h, K-value of the fish increased to 73% and gradually increased till the end. The K_1-value, G-value, P-value, and Fr-value were increased during storage time. The initial glycogen content in the individuals from the farm and live storage were 1.37 and 1.33 mg/g muscle. Significant differences in the plasma cortisol concentration were found in the fish after 24 and 72 h live storage compared to the fish caught from the farm. The L^* (lightness) value of the fish fillet from the farm, 24 h, and 72 h live storage was determined as 62.33, 58.12, and 56.21, respectively. After transporting the fish into the live seafood supply center, the total viable count was increased from 2.55 to 4.29 and 5.63 log CFU/ g. These results suggested that the measurement of quality indexes has a great potential for predicting the freshness of rainbow trout during live storage in seafood supply centers.

Keywords: Rigor mortis, K-value, Freshness, Live supply center

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Introduction

It is well recognized that fish are considerably more fragile than land animals, such as beef, pork, and lamb, and have a much faster spoilage process, which negatively affects muscle quality. The spoilage process begins instantly after death and continues until the fish muscle is inedible, and it is a complicated biochemical process affected by many factors, including endogenous muscle proteases and connective tissue and sarcoplasmic protein degradation (Liu *et al.,* 2021). As perishable food, fish experiences protein denaturation or degradation, lipid oxidation or hydrolysis and microorganism proliferation by the actions of endogenous enzymes and microorganisms during storage, suffering texture deterioration, off-odour, and shelf-life reduction (Shi *et al*., 2014; Zhu *et al.,* 2015).

Freshness is one of the most important factors that determine fish quality and is considered as the degree of various physical, chemical, biochemical and microbiological changes occurring post-mortem in fish (Franceschelli *et al.,* 2021). Assessment of rigor mortis offers a suitable method to show fish freshness at the early stages of storage, and fish in pre-rigor or full rigor are considered very fresh. After death, rigor mortis is triggered by the complete consumption of adenosine 5'-triphosphate (ATP) in muscle tissue and the rigor status can be determined simply by placing the fish on the table and observing tail drooping (Nimbkar *et al.,* 2021).

The K-value, which has been well accepted as a freshness index for fish consumed, was also proposed to indicate fish freshness. K-value is calculated by the ratio of ATP-related compounds, which is associated with rigor mortis. The concentrations of ATP and the products obtained from its breakdowns such as adenosine diphosphate (ADP), adenosine monophosphate (AMP), inosine monophosphate (IMP), hypoxanthine riboside (HxR), and hypoxanthine (Hx) are often calculated as a Hx-index, K-value or Ki-value for indicating fish freshness (Franceschelli *et al.,* 2021). The duration of pre-rigor period is essential in fish processing. The processing of fish can be performed before the onset of rigor mortis, which will finally lead to an increase in the yield and reduce the damage to fish flesh (Fan *et al.,* 2021).

A number of studies have shown that pre-rigor filleting causes a significant reduction in the incidence and severity of fillet gaping, improvement of visual color, and firmer texture (Brown *et al*., 2010; Aune *et al.,* 2014; Li *et al.,* 2015; Li *et al.,* 2017; Yang *et al.,* 2019; Fan *et al.,* 2021; Omwange *et al.,* 2022).

This study aimed to highlight the differences in quality changes between fish caught from the farm and a live supply center. Therefore, rigor mortis, K-value compounds, blood parameters, and water quality indices during live storage at 24h and 72h were investigated.

Materials and methods

Experimental design

The rainbow trout (*Oncorhynchus mykiss*, n=120, individual weight=300±3 g) was procured from a trout farm located in Alborz Province, Iran. 60 fish were brought to the laboratory under iced conditions in a polystyrene insulated container within 30 min under controlled temperature (-18ºC) and kept stored at -20±1ºC. Another 60 fish were transported alive to the live supply center within 30 min. In the live supply center, the fish were stored alive at 24 and 72 h. Then, the fish were sampled, headed, gutted, and filleted.

Rigor mortis (RM) measurement

The fish's rigor index is determined using the method described by (Bito *et al.*, 1983). Body and caudal fin flexion are used to calculate the rigor index. Half of the fish's length (head section) was placed on the table's edge. The highest between the table and the tail was then calculated. Based on the formula below, the results are presented as a percentage. Rigor-index (%) = [(L$_0$ − L)/L$_0$] × 100. L$_0$ is the vertical distance between a

fish's tail and the table's surface (it was immediately measured after fish death). L is the vertical distance between a fish's tail and the table's surface (for the designated time after the fish death).

Analysis of ATP-derived products and K-value calculation

ATP-related compounds were extracted and analyzed using an UltiMate 3000HPLC equipped with a RS Diode Array Detector and a Luna C18 column (5 µm, 100Å, 4.6 mm×250 mm) (Phenomenex, USA). A gradient elution with 0.8 mL/min flow rate was applied: 0 min, 100% 0.05 M PBS (pH=6.8); 5 min, 92% PBS+8% acetonitrile; 18 min, 92% PBS+8% acetonitrile; 20 min, 100% PBS. Column temperature was 35°C; injection volume was 20 µL. The samples were read at 254 nm. Standards (Sigma-Aldrich, US) were used for identifying and calculating the content of targeted compounds.

K-value, defined as the percentage of the amount of Ino and Hx to the total amount of ATP-related compounds (Saito *et al.,* 1959c):

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

Where ATP, ADP, AMP, IMP, HxR, and Hx represent adenosine-5′ - triphosphate, adenosine diphosphate, adenosine monophosphate, inosine 5'-

monophosphate, inosine and hypoxanthine, respectively. Inside relation degradation products of ATP are:

K$_1$-value (%) = [(Ino+Hx)/ (IMP+ Ino+ Hx)]× 100 (Karube *et al.,* 1984)
G-value (%) = [(Ino+Hx)] / (AMP+ IMP+ Ino)]× 100 (Burns *et al.,* 1985)

Fr-value (%) = [(IMP)/ (IMP+ HX+ Ino)× 100 (Gill *et al.,* 1987)

p-value (%) = [(Ino+HX)/ (AMP+ IMP +Ino+ Hx)]×100 (Shahidi *et al.* 1994)

Color determination

Color properties of fish fillet were evaluated using a CM-600d Colorimeter (Konica Minolta, Japan). The instrument was calibrated using standard white and black board. L* (brightness, 0 is black, 100 is white), a* (red-green value, positive value is red, negative value is green, 0 is neutral color), b* (yellow-blue value, positive value is yellow, negative value is blue and 0 is neutral). The values of ΔE* and browning index (BI) were calculated using following equations (Zhao *et al.,* 2020):

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$$

$$x = \frac{a^* + 1{,}75L^*}{5{,}645L^* + a^* - 3{,}012b^*}$$

$$BI\ (\%) = \frac{(x - 0{,}31)}{0{,}172} \times 100$$

Texture

Texture profile analysis was applied for textural properties investigation by using a texture analyzer (TA-XT. Plus, UK) equipped with a P/50 probe by following specifications: pressed depth 50%, test speed 1 mm/s, two 5 mm consecutive cycles with 5 s holding time in between. The whole experiment was performed at room temperature (21±1°C). Hardness, springiness and cohesiveness were calculated.

pH

The muscle pH were measured using a 206 digital pH meter (Testo AG, Germany) by inserting probe into muscle.

Protein solubility analysis

The protein solubility of the fillet sample was determined by the method of Benjakul and Bauer (2000). About 2 g of sample were homogenized with 18 mL of 0.6 M KCl. The homogenate was stirred (at 25–27°C) for 4 h, followed by centrifugation at 12000×g for 20 min at 4°C. To 10 mL of the supernatant, cold 50% (w/v) TCA was added to obtain the final concentration of 10%. The precipitate was washed with 10% TCA and solubilized in 0.5 M NaOH. Protein content was determined with the Biuret method (Robinson and Hogden, 1940):

$$Protein\ solubility\ (\%) = \frac{Protein\ Concentration\ in\ supernant}{Total\ Protein} \times 100$$

Plasma parameters assays

The osmolality of the plasma samples was measured in an automatic osmometer (model Type 15, Löser Messtechnik, Berlin, Germany) according to manufacturer's instructions. The concentrations of metabolites in plasma were determined spectrophotometrically using commercial kits for lactate (D-/L-Lactic acid, AK00141, NZYTech Ltd., Lisbon, Portugal), glucose (TR, GOD-POD, 61269/61270, Spinreatc, Girona, Spain). Cortisol was quantified using an

enzymatic immunoassay (RE52611, IBL International, Hamburg, Germany) measured in triplicates, following the recommendations provided by the manufacturer.

Microbial assay

The total viable count (TVC) was applied to microbial analysis during the storage period. 15 g of fish muscle was placed in a sterile bag containing 100 mL of 0.85% (w/v) sterile saline water. The mixture was agitated with a homogenizer for 1 min. The sample was subjected into the plate count agar medium which was incubated at 33 °C for 72 h. The number of colonies was counted and expressed as log CFU/ g.

Statistical analysis

Results were presented as mean values±standard deviation (SD). Data were analyzed using SPSS software (SPSS 17.0 for Windows, SPSS Inc.),

and analysis of variance (one-way ANOVA) was used to determine the difference between samples at a significance level of 0.05. For a significant difference, Turkey's multiple comparison test was used to investigate the difference between samples. Linear regression was used to explore the relationship between variables.

Results

Rigor mortis and ATP decomposition during rainbow trout storage

Rigor mortis changes and ATP decomposition were measured when samples arrived at the laboratory and the results are shown in Figure 1. Rigor started to increase at 12 h in the farm samples, indicating rigor onset, and reached a maximum level at 24 h in the farm samples, denoting full rigor. In live storage, rigor reached its highest level at 12 h and decreased at 72 h.
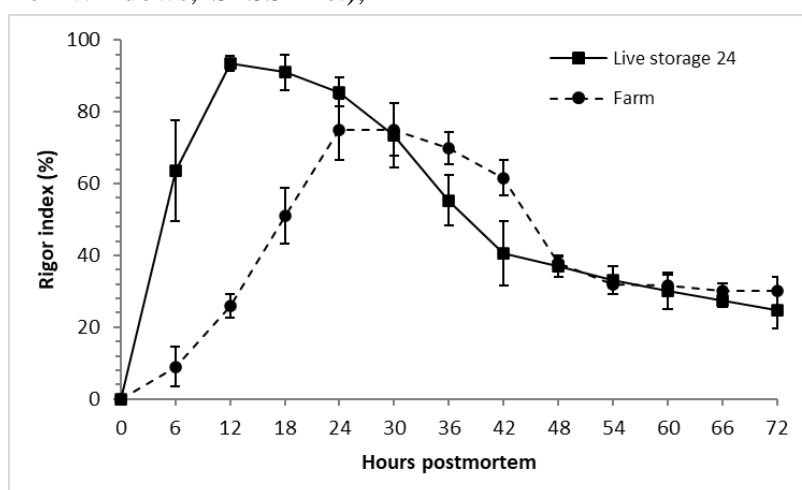

Figure 1: Changes in rigor mortis of rainbow trout.

Initial ATP content was 4.08 mmol/g at 24 h in live storage (Fig. 2). ATP declined to <0.1 mmol/g after 20 h and remained stable. ADP and AMP content

showed similar trends. IMP content of fish increased to a maximum (5.84 mmol/g) at 13 h and gradually decreased till the end. HxR increased constantly

and peaked at 72 h in the fish in live storage. Hx content kept increasing throughout storage and achieved 0.92 mmol/ g at 70 h, showing a slight decrease afterward. We analyzed all the five recommended indices for a better and biased-free understanding of spoilage and its progress in our experimental subjects. The initial K-value of fish in live storage was <8%. The K-value of fish climbed to >50% at 24 h. After 28 h, K-value of the fish increased to 73% and gradually increased till the end. K_1-value, G-value, P-value and Fr value increased during storage (Fig. 3).
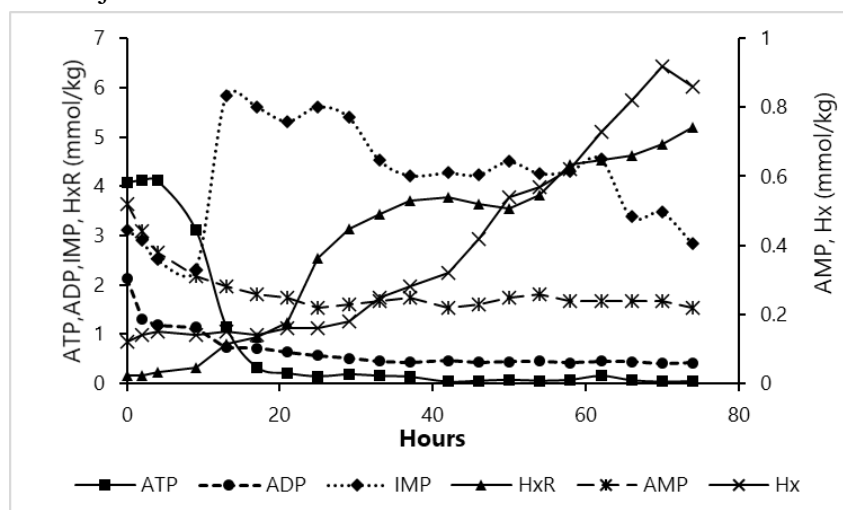

Figure 2: Changes in adenosine triphosphate (ATP) and its related compounds.
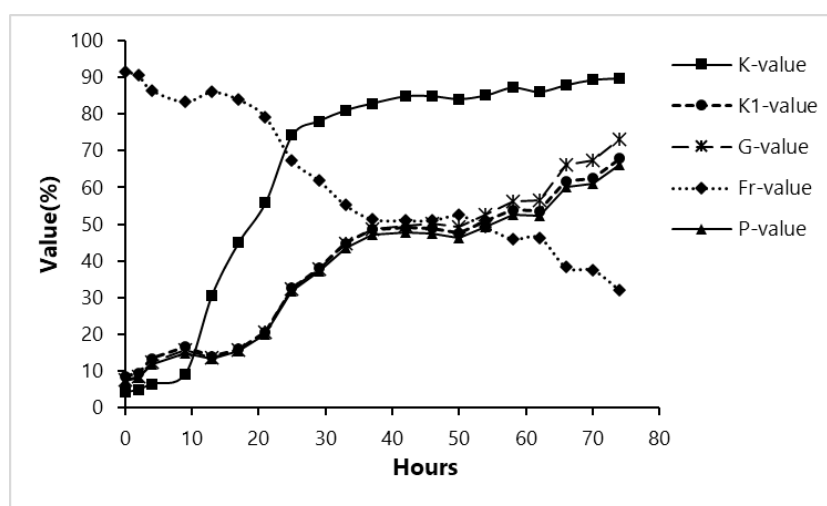

Figure 3: Changes in K-value, K_1 value, G-value, Fr-value, and P-value from rainbow trout samples.

Changes in the pH of fish muscle during storage at three times of storage (farm, 24 h, and 72 h in live storage) are shown in Figure 4.
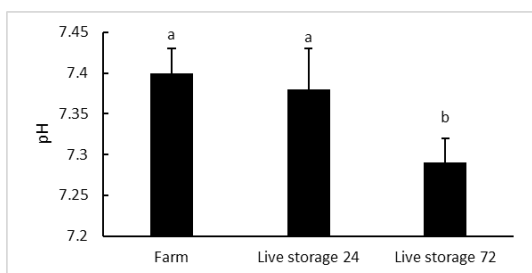
Figure 4: pH changes of rainbow trout fillets.

The initial pH of fish in the fish samples from the farm and 24 h in live storage was 7.39 and 7.36. Initial glycogen content in farm and live storage were 1.37 and 1.33 mg g^{-1} muscle. Both declined to <0.5 mg g^{-1} muscle in 72 h (Fig. 5), indicating a similar metabolic rate, and then the glycogen level stabilized. The pH of the fresh muscle was 7.38 in the fish farm samples and decreased sharply to the minimal level of 7.26 at 72 h in live storage.
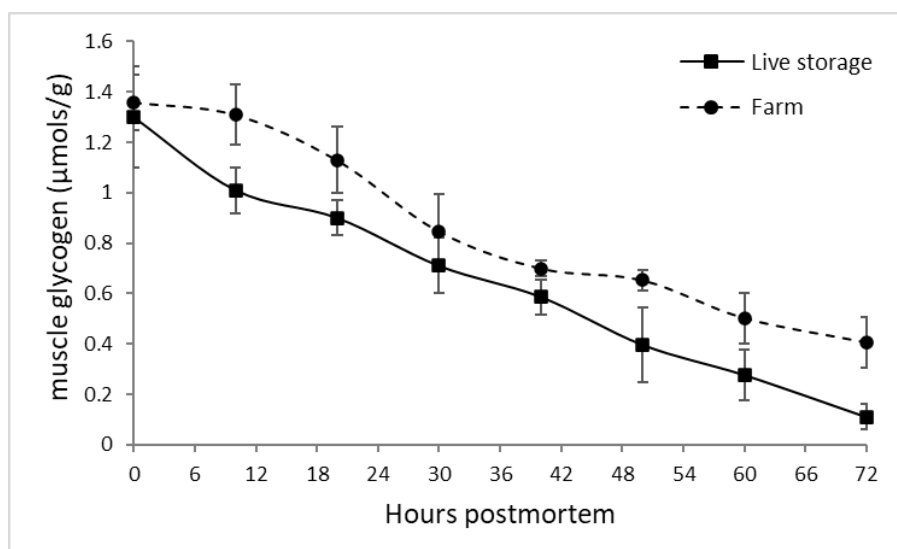

Figure 5: Glycogen in the muscle of rainbow trout .

In Figure 6, the initial protein solubility content of fish was 88.13 mgg^{-1} muscle and declined as storage time prolonged.

In Figure 7D fish osmolality was higher in live storage at 24 and 72 h compared to farm. After 72 h in live storage, the osmolality of fish was significantly higher than that in the farm and after 24 h in live storage and farm (p>0.05). Changes in plasma lactate concentration are displayed in Figure 7C. Lactate concentration increased in fish in live storage at 24 and 72 h, 4.14 and 10.98 mmol/L.

Changes in plasma glucose concentration are displayed in Figure 8A. Significant differences between groups were detected (p>0.05). A decrease in glucose levels after 24 and 72 h was displayed. Significant differences in plasma cortisol (Fig. 8B) concentration were found in fish after 24 and 72 h in live storage, where fish kept in live storage displayed higher levels when compared with fish in the fish captured from the farm.
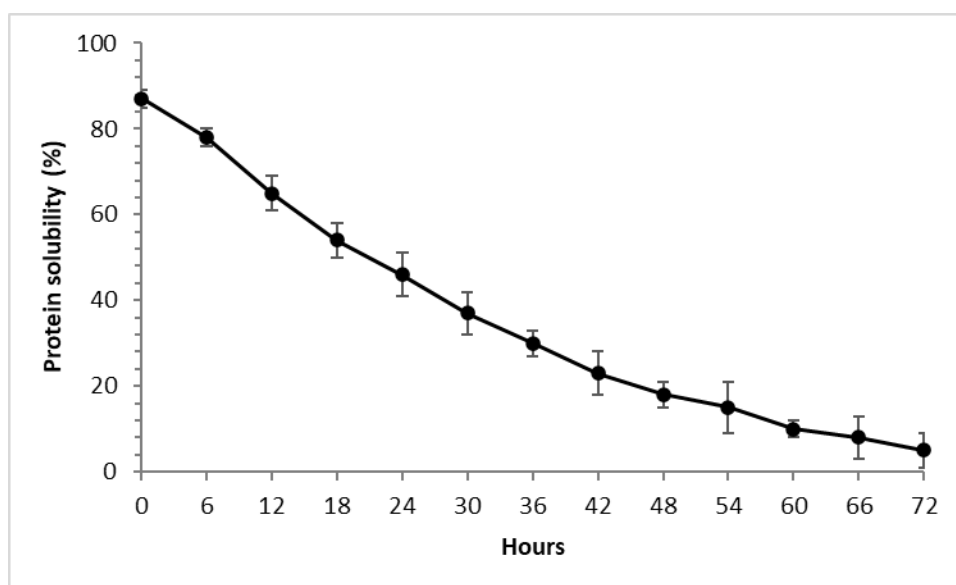
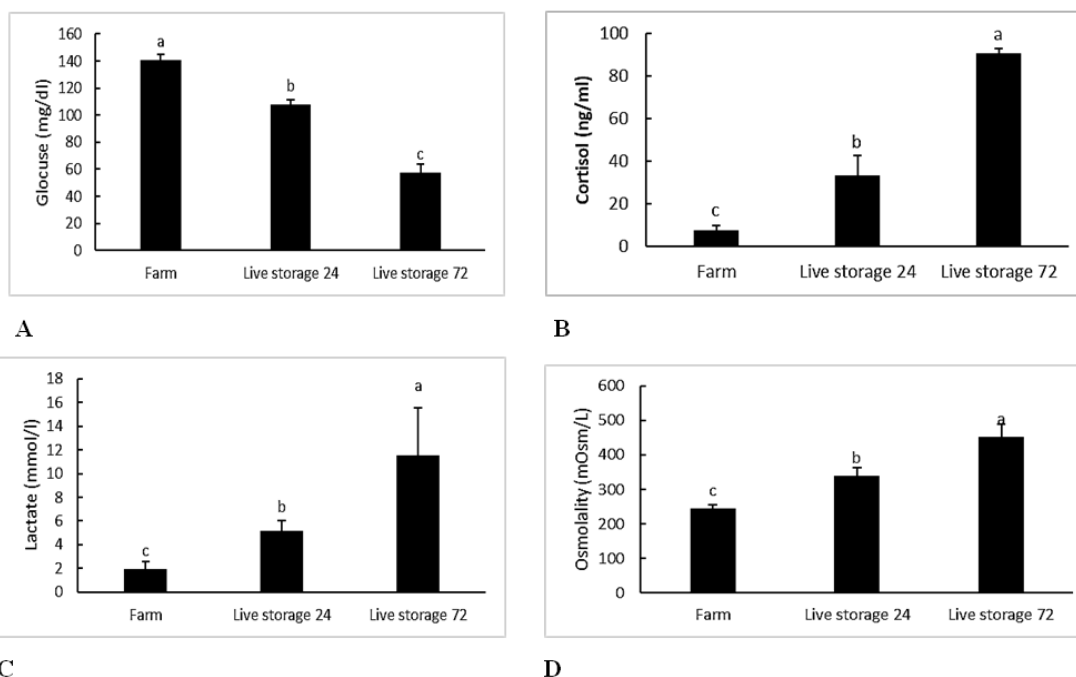**Figure 6:** Protein solubility changes of rainbow trout.

Figure 7: Mean± SE plasma cortisol, lactate, glucose, and osmolality in rainbow trout at the farm and after storage in live supply seafood center.

Changes in the water quality parameters among different groups are presented in Figure 8. Phosphate, nitrite, and nitrate levels gradually increased.

The initial pH of water on the farm was 7.87. However, the pH was decreased to 7.45 and 6.51 after 24 and 72 h storage in the live fish center. For phosphate content, significant differences ($p<0.05$) were found between the 24 and 72 h live storage. No significant differences ($p>0.05$) were found in the nitrate level. In this study, it was observed that after 72 h live storage, the nitrite concentration was significantly increased ($p<0.05$).
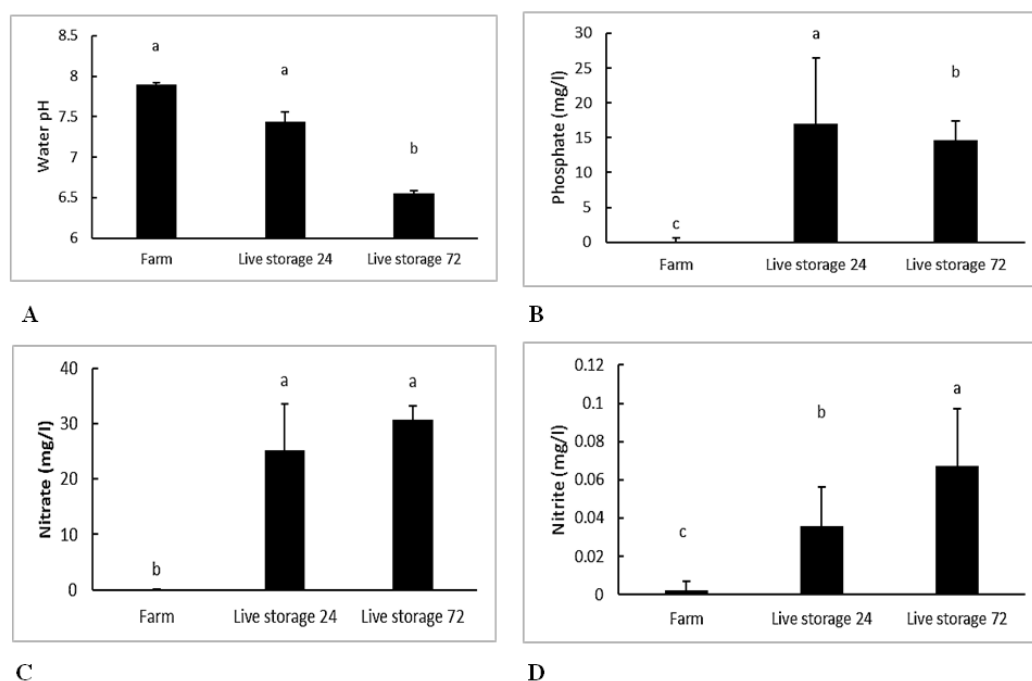
Figure 8: Changes in the water quality variables.

Color measurements of rainbow trout fillets in storage in different conditions are shown in Table 1. In the beginning of trial, the L^* (lightness) values of the farm, 24 and 72 h storage were determined as 62.33, 58.12 and 56.21, respectively. The a^*(+ red or - green) value of the fish fillet from the farm was changed from -1.07 to -0.65 and -0.41 after 24 and 72 h live storage, respectively. According to b^* (+ yellow or - blue)

values, significant differences were determined between 24 and 72 h. For ΔE, values ranged from 5.098 to 6.084, respectively. BI significantly increased with the increased storage.

Texture changes are shown in Table 1. It was determined that storage conditions affect texture qualities (hardness, springiness, and cohesiveness). Changes in TVC in rainbow trout are shown in Table 1.

Table1: Changes in color properties (L*, a*, b* value), texture properties (Hardness, Springiness, Cohesiveness), and total viable count (TVC, log CFU/g) of rainbow trout fillet.

Indexes	Farm	24h	72h
L^*	62.33±2.33[a]	58.12±1.55[b]	56.21±2.14[c]
a^*	-1.07±0.11[a]	-0.65±0.07[b]	-0.41±0.24[c]
b^*	14.33±1.66[a]	14.75±2.23[b]	14.98±1.55[c]
ΔE	5.098±0.41[a]	6.084±0.74[b]	4.600±0.33[c]
BI	26.25±0.26[a]	29.77±0.54[b]	31.73±0.11[c]
Hardness (g)	11.75±1.11[a]	11.81±1.16[b]	14.33±1.24[c]
Springiness (%)	0.23±0.01[a]	0.24±0.02[b]	0.27±0.01[c]
Cohesiveness (%)	0.21±0.02[a]	0.22±0.01[b]	0.24±0.03[c]
TVC	2.55±0.06[a]	4.29±0.08[b]	5.63±0.03[c]

Lowercase letters in different rows indicate the significantly different levels ($p<0.05$).

After transporting the fishes into live storage, the TVC of rainbow trout was increased from 2.55 to 4.29 and 5.63 log CFU/g. The bacterial count increased according to the time of storage. Significant differences were observed between the groups at the end of storage ($p<0.05$).

Discussion

The stiffening of muscle that appears shortly after death is known as rigor mortis. Fish generally exhibit rigor mortis starting from about 1–6 h after death although the onset, intensity and resolution of rigor depend on several factors such as species, size, stress, killing method, catching conditions, handling and post-mortem and temperature, among others (Erikson *et al.,* 2018). Handling of fish once rigor has started is not recommended because the yield of fillets is poorer and the handling may cause gaping (Sigholt *et al.,* 1997; Lyu *et al*., 2017). The stress associated with transport, crowding, and handling subsequent effects on welfare, muscle pH, and development of rigor times have been reported in a broad range of different farmed fish species (Brown *et al.,* 2010; Roth *et al.,* 2009; Roth *et al.,* 2012). The delay in the onset and resolution of rigor mortis extends the shelf life of the fish (Roth *et al.,* 2006). In a study by Fan *et al*. (2021), rainbow trout fillet, gradually turned to rigor in 3 h of storage. The rigor mortis index decreased from 14.83 to 2.50 cm. Full rigor mortis was reached at about 5h showing 1.90 cm and the rigidness of the fish continued to increase to 9 h of storage. In the present study, <60% of ATP in the muscle of fish had been degraded during transportation (Fig. 2). Our results showed that the increase in rigor mortis was faster for storage at 24 h. This was likely due to the solidification of lipids in the body of the fish and this added to the firmness build-up. Results of rainbow trout and salmon have been reported based on the crowding and pumping procedures of pre-slaughter status, therefore, rigor mortis was detected in different death time, from 3 to 12 h and accompanied by a different reduction in rigidness (Merkin *et al.,* 2010, 2014). Full rigor in *Hypophthalmichthys molitrix* and *Ctenopharyngodon idellus* stored at 4°C, was found to occur at 48 and 24h.

Rigor mortis starts in different fish muscles according to the depletion of ATP and progressively extends to the whole fish (Li *et al.,* 2017). The fish contained 4.03±0.03 mmol/ kg of ATP after delivery to the laboratory. ATP in the rainbow trout was considerably degraded after death as the muscle contained a certain 2.21±0.08 mmol/kg of ADP and a relatively large IMP concentration (3.02±0.05 mmol/ kg). The ATP content decreased to nearly 18 h of storage, which was much later than the occurrence of rigor mortis. The result showed that degradation into ADP and decomposition into IMP in the rainbow trout muscle was fast that meant the ATPase and AMP deaminase activities were quite positive after the death. ATP and its derived compound were analyzed during fish storage as indicated in Figure 3. The prominent compound detected at

an early storage stage was IMP, showing a maximum content of 5.58±0.06 mmol/kg in 16 h. From 16 to 74 h, its content remained high 2.81±0.03 mmol/kg, with a very decrease during storage. In accordance with the decrease in IMP, HxR content increased. HxR was slowly converted into Hx. The initial K-value was 5.11±0.05% and increased gradually with storage reaching 89.91±0.81% in 72 h. It has been recognized that a K-value <10% is acceptable for instantly killed fish. Our results were consistent with previously reported K-values in rainbow trout (Chytiri *et al.,* 2004).

Maximum K-values at the rejection of the fish have been reported in several species ranging from 80% in European whitefish (*Coregonus wartmanni*) (Hattula *et al.* 1993); 70–80% in Atlantic salmon (*Salmo salar*) (Erikson *et al.*, 1997); 60–70% in farmed seabass (*Dicentrarchus labrax*) (Tejada *et al.*, 2006), farmed turbot (*Psetta maxima*) (Rodriguez *et al.* 2006) and wild turbot (*Scophthalmus maximus*) (Özogul *et al.*, 2006); to 30–45% in hoki (*Macruronus novaezelandiae*) (Ryder *et al.* 1993), farmed gilthead seabream (*Sparus aurata*) (Tejada *et al.*, 2006), and Senegalese sole (*Solea senegalensis*) (Tejada *et al.*, 2007). Cold-water fishes tend to show an increase in K-value, whereas warm-water species show a slow increase in K-value (Uchiyama *et al.,* 1966). The K-value of red sea bream (Fuentes *et al.,* 2013) increased to 30% over 25 days, horse mackerel increased to 40% over 14 days, flounder increased to 70% within 7 days and the K-value

increase obtained in the present study was a little slower than that of cod and much faster than that of sea bream (Yuan *et al.,* 2018; Yoshioka *et al.,* 2019). K_1-value of 20% has been defined by researchers as the limit for raw fish consumption. In farmed fish, the period ranged between 3 and 4 days in sea bass, 7 and 8 days in sea bream and 8 and 10 days in Senegalese sole, depending on the batch studied (Huidobro *et al.* 2001; Tejada *et al.* 2006a). Differences had been found in reaching this value depending on the method of storage. K-value of 20% was measured in farmed turbot stored in slurry ice at 10 days of storage; however, in the fish stored in flake ice, the K-value increased faster (Piñeiro *et al.* 2005).

In salmonids, the K-value seems to increase sharply during the first days of storage before leveling off at about day 7th post-mortem. However, the variation in values reported for salmonids seems to be large, with K-value after seven days of storage ranging between 40 and 80%. Handling conditions, slaughtering methods, and physical parameters of water affect the rigor mortis and then K-value (Erikson *et al.*, 1997; Shumilina *et al.,* 2016). Higher K, K_1, G, and P values correspond to a decrease in the freshness of the fish (Alasalvar *et al.,* 2002). All these values reduce variability compared to former methods such as determination of hypoxanthine or free ribose concentration and are better correlated with the freshness of the fish. K-value <60% was recognized as commercial fish processing (Yuan *et al.,* 2018), in the present result when the fish samples

were stored for less than 23h, the K-value <60%. Different stages of rigor mortis influence the solubility of protein. The protein solubility of the samples stored on the farm was found 88.13% per rigor stage of the fish. After that, solubility decreased continuously throughout the in-rigor and post-rigor stages. Protein solubility of silver jewfish, Ribbon fish, and Bombay duck was determined during ice storage by Hossain *et al*. (2020), which is quite similar to the results obtained in the present study. The myofibrillar protein solubility decreased continuously with the progress of the storage period.

Changes in plasma parameters were observed by Jensen *et al*. (1998) in seabass. In our study plasma parameters were modulated differently by the transporting and live storage. This period (24 and 72h) was characterized by decreased availability and use of glucose but increased lactate and cortisol. Also, an increase in plasma osmolality levels at the beginning of the acclimation was observed in fish transferred from farm to live storage. The study of Silva-Brito *et al*. (2019) did not observe changes in the levels of plasma glucose after 24 h, although lactate levels were significantly increased after transporting. In this regard, fish also appear to use lactate as an energy substrate during storage acclimation. Higher cortisol levels were observed in the fish of live storage, which suggests that live storage at 24 and 72h is evoking a general stress response in fish.

L^* value of the farm was slightly higher than that of 24h and 72h after live storage, indicating its higher lightness. Reduction in L^* value was found in mirror, silver, and common carp (Zhu *et al.,* 2015; Hao *et al.,* 2020; Shi *et al.,* 2020). Both a^* and b^* values increased during live storage and a significantly different between them was observed. Our results suggest an increase in redness and yellowness of fish muscle during storage. Protein and lipid oxidation were reported to strengthen the yellowness of muscle by oxidation products such as peroxides, malondialdehyde, and free fatty acids, which can modify the absorption of light (Martinez *et al.,* 2011). BI is an essential criterion for food acceptability during storage. The BI reflects the degree of glycosylation (Zhao *et al.,* 2020). Therefore, both glycosylation and non-glycosylation could change the color of muscle.

Textural properties in fish are influenced by several factors including species, age and size, fat content, and distribution and proteases (Chan *et al.,* 2020). Fish storage causes damage to tissue structure and muscle membrane, which results in a decrease in muscle hardness. The declined pH could destroy the sarcoplasmic reticulum by releasing endogenous to hydrolysis myofibril structure, resulting in soft texture (Wang *et al*., 2022; Kilinc *et al.,* 2009). The initial hardness in the farm was 11.75g, then increased to 11.81 and 14.33g after 24h and 72h. Compared with the fish captured from the farm, fish storage in 24 and 72h, was more exposed to

microorganisms, which might promote the degradation of muscle protein via exogenous enzymes, resulting in a soft and less elastic texture.

Initial TVC was 2.55 log CFU/g in farm samples, lower than that reported by Hao *et al*. (2020) and Li *et al*. (2017), suggesting the good sanitary status of the fishes. TVC increased as storage time extended. After 72 h, the TVC of fish increased to 5.63 log CFU/ g, higher than that of 24h, 4.29 log CFU/g. Neither exceeded the maximum acceptable level of TVC for fish (7.0 log CFU/g). Muscle protein degradation could enable the release of more soluble nutriens and promote microorganism growth.

In conclusion, results from this study suggest interactions between the physical parameters of water and fish quality characteristics and blood factors. There were differences in rigor mortis, K-value compounds, ATP and its derived compound, plasma factors, TVC, color, and texture between farm, 24h and 72h at storage. As storage extended, fish kept live storage at 24h, exhibited slightly higher preservatives and better muscle quality than 72h. Results showed that the rapid consumption of ATP induced accelerated conversation of xanthine dehydrogenase to xanthine oxidase.

References

Alasalvar, C., Taylor, K.D.A. and Shahidi, F., 2002. Comparative quality assessment of cultured and wild seabream (*Sparus aurata*) stored in ice. *Journal of Agricultural and Food Chemistry*, 50, 2039–2045. DOI:10.1021/jf010769a

Aune, T.F., Olsen, R.L., Akse, L., Ytterstad, E. and Esaiassen, M., 2014. Influence of different cold storage temperatures during the rigor mortis phase on fillet contraction and longer-term quality changes of Atlantic cod fillets. *LWT-Food Science and Technology*, 59(**1**), 583–586. DOI:10.1016/j.lwt.2014.04.018.

Benjakul, S. and Bauer, F., 2000. Physicochemical and enzymatic changes of cod muscle proteins subjected to different freeze– thaw cycles. *Science and Food Agriculture*, 80(**8**), 1143–50. DOI:10.1002/1097-0010 (200006)80:8<1143:AID-JSFA610>3.0.CO; 2-C.

Bito, M., Yamada, K., Mikumo, Y. and Amano, K., 1983. Studies on rigor mortis of fish-I. Difference in the mode of rigor mortis among some varieties of fish by modified cutting's method. *Bulletin Tokai Fisheries Research Lab*, 109, 89–96.

Brown, J.A., Watson, J., Bourhill, A. and Wall, T., 2010. Physiological welfare of commercially reared cod and effects of crowding for harvesting. *Aquaculture*, 298, 315–324. DOI:10.1016/j.aquaculture.2009.10.028

Burns, G.B., Kee, P.J. and Irvine, B.B., 1985. Objective procedure for fish freshness evaluation based on nucleotide changes using a HPLC system. *Canadian Technical Report*

of Fisheries and Aquatic Sciences No. 1373, pp. 95-112.

Chan, S.S., Roth, B., Skare, M., Hernar, M., Jessen, F., Lovdal, T., Jakobsen, A.N. and Lerfall, J., 2020. Effect of chilling technologies on water holding properties and other quality parameters throughout the whole value chain: Farm whole fish to cold-smoked fillets of Atlantic salmon (*Salmo salar*). *Aquaculture*, 526, 735381. DOI:10.1016/j.aquaculture.2020.735 381.

Chytiri, S., Paleologos, E., Savvaidis, I. and Kontominas, M.G., 2004. Relation of biogenic amines with microbial and sensory changes of whole and filleted freshwater rainbow trout (*Onchorynchus mykiss*) stored on ice. *Journal of Food Protection*, 67(**5**), 960–965. DOI:10.4315/0362-028X-67.5.960.

Erikson, U., Bayer, A.R. and Sigholt, T., 1997. Muscle high-energy phosphates and stress affect K-values during ice storage of Atlantic salmon (*Salmo salar*). *Journal of Food Science,* 62(**1**), 43–47. DOI:10.1111/j.1365-2621.1997.tb04365.x

Erikson, U., Shabani, F., Beli, E., Muji, S. and Rexhepi, A., 2018. The impacts of perimortem stress and gutting on quality index and colour of rainbow trout (*Oncorhynchus mykiss*) during ice storage: a commercial case study. *European Food Research and Technolog*y, 244, 197-206. DOI:10.1007/s00217-017-2944-9

Fan, X., Jin, Z., Liu, Y., Chen, Y., Konno, K. and Zhu, B., 2021. Effects of super-chilling storage on shelf-life and quality indicators of *Coregonus peled* based on proteomics analysis. *Food Research International*, 143, 110229. DOI:10.1016/j.foodres.2021.110229.

Fan, X., Lin, X., Wu, Ch., Zhang, N., Cheng, Q., Qi, H., Konno, K. and Dong, X., 2021. Estimating freshness of ice storage rainbow trout using bioelectrical impedance analysis. *Food Science and Nutrition*, 9, 154-163. DOI:10.1002/fsn3.1974.

Franceschelli, L., Berardinella, A., Dabbou, S., Ragni, L. and Tartagni, M., 2021. Sensing Technology for Fish Freshness and Safety: A Review. *Sensors*, 21(**4**), 1373. DOI:10.3390/s21041373.

Fuentes, A., Masot, R., Fernández-Segovia, I., Ruiz-Rico, M., Alcaniz, M. and Barat, J.M., 2013. Differentiation between fresh and frozen-thawedsea bream (*Sparus aurata*) using impedance spectroscopy techniques. *Innovative Food Science & Emerging Technologies*, 19, 210–217. DOI:10.1016/j.ifset.2013.05.001

Gill, T.A., Thompson, J.W., Gould, S. and Sherwood, D., 1987. Characterization of quality deterioration of yellowfin tuna. *Journal of Food Science*, 52, 580–583. DOI:10.1111/j.1365-2621.1987.tb06679.x

Hao, R., Pan, J., Tilami, S.K., Shah, B.R. and Mraz, J., 2020. Post-mortem quality changes of common

carp (*Cyprinus carpio*) during chilled storage from two culture systems. *Journal of Sciences of Food and Agriculture*. 100, 91-100. DOI:10.1002/jsfa.10618.

Hattula, T., Kiesvaara, M. and Moran, M., 1993. Freshness evaluation in European white fish (*Coregonus wartmanni*) during chill storage. *Journal of Food Science*, 58(**6**), 1212–1215. DOI:10.1111/j.1365-2621.1993.tb06150.x

Hossain, I., Shikha, F.H. and Yeasmin, S., 2020. Changes in gel forming ability and protein solubility of pangasius (*Pangasianodon hypophthalmus*) muscle at different rigor stages during storage at room temperature (28 to 32°C) and in ice. *Bangladesh Journal of Fisherie*s, 32(**2**), 313-322. DOI:10.52168/bjf.2020.32.36

Huidobro, A., Pastor, A. and Tejada, M., 2001. Adenosine triphosphate and derivatives as freshness indicators of gilthead sea-bream (*Sparus aurata*). *Food Science and Technology International*, 7(**1**), 23–30. DOI:10.1106/B69Q-LXFQ-HPQP-9VX7

Jensen, M.K., Madsen, S.S. and Kristiansen, K., 1998. Osmoregulation and salinity effects on the expression and activity of Na$^+$, K$^+$-ATPase in the gills of European sea bass, *Dicentrarchus labrax* (L.). *The Journal of Experimental Zoology*, 282, 290–300. DOI:10.1002/(SICI)1097-

010X(19981015)282:3<290::AID-JEZ2>3.0.CO;2-H

Karube, I., Matsuoka, H., Suzuki, S., Watanabe, E. and Toyama, K., 1984. Determination of fish freshness with an enzyme sensor system. *Journal of Agricultural and Food Chemistry*, 32, 314–319. DOI:10.1021/jf00122a034

Kilinc, B., Cakli, S., Dincer, T. and Tolasa, S., 2009. Microbiological, chemical, sensory, color, and textural changes of rainbow Trout fillets treated with sodium acetate, sodium lactate, sodium citrate, and stored at 4°C. *Journal of Aquatic Food Product Technology*, 18, 3-17. DOI:10.1080/10498850802580924.

Li, K.F., Luo, Y.K. and Shen, H.X., 2015. Postmortem changes of Crucian carp (*Carassius auratus*) during storage in ice. *International Journal of Food Properties*, 18(**1**), 205–212. DOI:10.1080/10942912.2011.614986.

Li, Q., Zhang, L.T., Han, L., Song, S.J. and Luo, Y.K., 2017. Comparison of postmortem changes in ATP-related compounds, protein degradation and endogenous enzyme activity of white muscle and dark muscle from common carp (*Cyprinus carpio*) stored at 4°C. LWT - *Food Science and Technology*, 78, 317–324. DOI:10.1016/j.lwt.2016.12.035.

Liu, J., Yang, F., Gao, P., Yu, P., Jiang, Q., Xu, Y. and Xia, W., 2021. The impact of crucial protein degradation in intramuscular

connective tissue on softening of ice-stored grass carp (*Ctenopharyngodon idella*) fillets. *Food Science and Technology*, 55, 101-111. DOI:10.1111/ ijfs.14979.

Lyu, J., Li, Q., Zhang, L., Zhang, J., Dong, Z., Feng, L. and Luo, Y., 2017. Changes in quality of rainbow trout (*Oncorhynchus mykiss*) fillets preserved with salt and sugar at low concentrations and stored at 4 degrees C. *International Journal of Food Properties*, 20(10), 2286–2298. DOI 10.1080/10942912.2016.1236272.

Martinez, I, Wang, P.A., Slizyté, R., Jorge, A., Dahle, S.W., Cañas, B., Yamashita, M., Olsen, R.L. and Erikson U., 2011. Protein expression and enzymatic activities in normal and soft textured Atlantic salmon (*Salmo salar*) muscle. *Food Chemistr*y, 126, 140-148. DOI:10.1016/j.foodchem.2010.10.090

Merkin, G.V., Roth, B., Gjerstad, C., Dahl-Paulsen, E. and Nortvedt, R., 2010. Effect of pre-slaughter procedures on stress responses and some quality parameters in sea-farmed rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, 309, 231-235. DOI:10.1016/j.aquaculture.2010.08.025.

Merkin, G.V., Stien, L.H., Pittman, K. and Nortvedt, R., 2014. The effect of stunning methods and season on muscle texture hardness in Atlantic salmon (*Salmo salar* L.). *Journal of Food Sciences*, 79, 37-41. DOI:10.1111/1750-3841.12473.

Nimbkar, S., Auddy, M., Manoj, I. and Shanmugasunsaram, S., 2021. Novel Techniques for Quality Evaluation of Fish: A Review. *Food Reviews International*, 84, 195-208. DOI:10.1080/87559129.2021.1925291.

Omwange, K.A., Saito, Y., Riza, D.F.A., Zichen, H., Kuramoto, M., Shiraga, K., Kondo, N. and Suzuki, T., 2022. Japanese dace (*Tribolodon hakonensis*) fish freshness estimation using front-face fluorescence spectroscopy coupled with chemometric analysis. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 276, 121209. DOI:10.1016/j.saa.2022.121209.

Özogul, Y., Özogul, F., Kuley, E., Özkutuk, A.S., Gökbulut, C. and Köse, S., 2006. Biochemical, sensory and microbiological attributes of wild turbot (*Scophthalmus maximus*), from the Black Sea during chilled storage. *Food Chemistry*, 99, 752–758. DOI:10.1016/j.foodchem.2005.08.053

Piñeiro, C., Bautista, R., Rodríguez, O., Losada, V., Barros-Velaázquez, J. and Aubourg, S.P., 2005. Quality retention during the chilled distribution of farmed turbot (*Psetta maxima*): effect of a primary slurry ice treatment. *International Journal of Food Science & Technology*, 40, 817–824. DOI:10.1111/j.1365-2621.2005.00989.x

Robinson, H.W. and Hogden C.G., 1940. The Biuret Reaction in the

Determination of Serum Proteins. *Annals of clinical and Laboratory Science*, 135, 707–25. DOI:10.1016/s0021-9258(18)73135-9

Rodriguez, O., Barros-Velazquez, J.M., Piñeiro, C., Gallardo, J.M. and Aubourg, S.P., 2006. Effects of storage in slurry ice on the microbial, chemical and sensory quality and on the shelf-life of farmed turbot (*Psetta maxima*). *Food Chemistry*, 95(**2**), 270–278. DOI:10.1016/j.foodchem.2004.11.054

Roth, B., Slinde, E. and Arildsen, J., 2006. Pre and post mortem muscle activity in Atlantic salmon (*Salmo salar*). The effect on rigor mortis and the physical properties of flesh. *Aquaculture*, 257, 504–510. DOI:10.1016/j.aquaculture.2005.10.021

Roth, B., Birkeland, S. and Oyarzun, F., 2009. Stunning, pre slaughter and filleting conditions of Atlantic salmon and subsequent effect on flesh quality on fresh and smoked fillets. *Aquaculture*, 289, 350–356. DOI:10.1016/j.aquaculture.2009.01.013

Roth, B., Grimsb, E., Slinde, E., Foss, A., Stien, L.H. and Nortvedt, R., 2012. Crowding, pumping and stunning of Atlantic salmon, the subsequent effect on pH and rigor mortis. *Aquaculture*, 326-329, 178-180. DOI:10.1016/j.aquaculture.2011.11.005.

Ryder, J.M., Fletcher, G.C., Stec, M.G. and Seelye, R.J., 1993. Sensory, microbiological and chemical changes in hoki stored in ice. *International Journal of Food Science & Technology*, 28, 169–180. DOI:10.1111/j.1365-2621.1993.tb01262.x

Saito, T., Arai, K. and Matsuyoshi, M., 1959c. A new method for estimating the freshness of fish. *Bulletin of the Japanese Society of Scientific Fisheries*, 24(**9**), 749–750. DOI:10.2331/suisan.24.749.

Shahidi, F., Chong, X. and Dunajski, E., 1994. Freshness quality of harp seal (*Phoca groenlandica*) meat. *Journal of Agricultural and Food Chemistry*, 42, 868–872. DOI:10.1021/jf00040a005

Shi, C., Cui, J., Yin, X., Luo, Y. and Zhou, Z., 2014. Grape seed and clove bud extracts as natural antioxidants in silver carp (*Hypophthalmichthys molitrix*) fillets during chilled storage: Effect on lipid and protein oxidation. *Food Control*, 40, 134-139. DOI: 10.1016/j.foodcont.2013.12.001

Shi, L., Yin, T., Wang, L., Xiong, G., Gao, R., Ding, A., Li, X., Wu, W., Qiao, Y. U., Liao, L. I. and Jiao, C., 2020. Effect of pre-chilling time on the physicochemical properties of channel catfish during frozen storage. *International Journal of Refrigeration*, 115, 56–62. DOI: 10.1016/j.ijrefrig.2020.01.015

Shumilina, E., Slizyte, R., Mozuraityte, R., Dykyy, A., Stein, T.A. and Dikiy, A., 2016. Quality

changes of salmon by-products during storage: assessment and quantification by NMR. *Food Chemistry*, 211, 803-811. DOI:10.1016/j.foodchem.2016.05.088.

Sigholt, T., Erikson, U., Rustad, T., Johansen, S., Nordtvedt, T.S. and Seland, A., 1997. Handling stress and storage temperature affect meat quality of farmed-raised Atlantic salmon (*Salmo salar*). *Food Science*, 62, 898–905. DOI:10.1111/j.1365-2621.1997.tb15482.x

Silva-Brito, F., Timóteo, F., Esteves, A., Peixoto, M.J., Ozorio, R. and Magnoni, L., 2019. Impact of the replacement of dietary fish oil by animal fats and environmental salinity on the metabolic response of European Seabass (*Dicentrarchus labrax*). *Comparative Biochemistry and Physiology, Part B*, 233, 46-59. DOI:10.1016/j.cbpb.2019.04.004.

Tejada, M., Huidobro, A. and Fouad Mohamed, G., 2006. Evaluation of two quality indices related to ice storage and sensory analysis in farmed gilthead seabream and seabass. *Food Science and Technology International*, 12(3), 261–268. DOI:10.1177/1082013206065955

Tejada, M., De las Heras, C. and Kent, M., 2007. Changes in the quality indices during ice storage of farmed Senegalese sole (*Solea senegalensis*). *European Food Research and Technology*, 225, 225-232. DOI:10.1007/s00217-006-0407-9.

Uchiyama, H., Suzuki, T., Ehira, S. and Noguchi, E., 1966. Studies on relation between freshness and biochemical changes of fish muscle during ice storage. *Nippon Suisan Gakkaishi*, 32(**1**), 280–285. DOI:10.2331/suisan.32.280

Wang, H., Zheng, Y., Shi, W. and Wang, X., 2022. Comparison of Arrhenius model and artificial neuronal network for predicting quality changes of frozen tilapia (*Oreochromis niloticus*). *Food Chemistry*, 372, 131268. DOI:10.1016/j.foodchem.2021.131268.

Yang, F., Jia, S.N., Liu, J.X., Gao, P. and Zhan, X.B., 2019. The relationship between degradation of myofibrillar structural proteins and texture of super-chilled grass carp (*Ctenopharyngodon idella*) fillet. *Food Chemistry*, 301, 125278. DOI:10.1016/j.foodchem.2019.125278.

Yoshioka, T., Konno, Y. and Konno, K., 2019. Below-zero storage of fish to suppress loss of freshness. *Fisheries Science*, 85(**3**), 601–609. DOI:10.1007/s12562-019-01294-3

Yuan, P., Wang, Y., Miyazaki, R., Liang, J., Hirasaka, K., Tachibana, K. and Taniyama, S., 2018. A convenient and nondestructive method using bio-impedance analysis to determine fish freshness during ice storage. *Fisheries Science*,

84(**6**),   1099–1108. DOI:10.1007/s12562-018-1256-8

Zhao, H., Kang, X., Zhou, X., Tong, L., Yu, W., Zhang, J., Yang, W., Lou, Q. and Huang, T., 2020. Glycosylation fish gelatin with gum Arabic. *Functional and structural properties*. 139, 110634. DOI:10.1016/j.lwt.2020.110634

Zhu, S., Zhou, Z., Feng, L. and Luo, Y., 2015. Postmortem changes in physicochemical properties of songpu mirror carp (*Cyprinus carpio*) during iced storage. *Food Bioscience*, 9,   75-79. DOI:10.1016/j.fbio.2014.12.003