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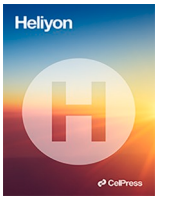

Research article

A solution for fillet quality: Slaughter age's effect on protein mechanism and oxidation

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ABSTRACT

Physico-chemical properties of fish flesh are reliable predictors of fillet quality and nutritional value. In our study, the age-related variations of the chemical composition, pH, water activity (aw), water holding capacity (WHC), color and texture analysis, protein thermal stability, myofibrillar fragmentation index (MFI), glycogen content, protein oxidation and protein profiles were investigated in *Oncorhynchus mykiss* (rainbow trout) fillet. The results revealed that protein denaturation temperatures ($T_{max}1$ and $T_{max}2$) decreased by 2 % and 11.6 % depending on fish age. $T_{max}1$ and $T_{max}2$ values in the same groups were raised 71 % at 11 months' fish and this increase was 58 % at 23 months' fish. An age-related reduction by 66.6 % and 31.25 % was noticed for protein oxidation markers sulfhydryl groups and disulfide bonds. MFI value increased by 86.6 % connected with age.

The characteristics of fish meat quality are complex and are influenced by various factors that affect the degree of freshness of the product and its acceptance in the market. Taking into account the different demands of the consumer, this study has shown that age at slaughter has an impact on final product quality and that the recommended age at slaughter, taking into account market weight, positively affects meat quality.

1. Introduction

Micronutrient composition as well as protein quality and polyunsaturated fatty acids (PUFA) are important in consumer health and

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preference. Chemical and nutritional properties of seafood provide high reliable way to assess fillet quality. In addition to its high protein and low carbohydrate content, seafood products contain essential amino acids, omega-3 fatty acids, vitamins, mineral substances and have a low cholesterol/calorie value, which increases their importance in a balanced diet day by day. The chemical composition of fish is very close to that of other terrestrial animals. The main components of fish are moisture (66–88 %), protein (15–24 %), fat (0.1–24 %) and ash (0.8–2%). In terms of carbohydrates, fish contains no more than 0.3 % glycogen and 1–2% minerals, 0.5 % calcium, 0.25 % phosphorus and 0.1 % vitamins A, B, C and D in addition to their vitamins in fat [1]. Fish protein has a stable composition of essential amino acids with higher lysine and lower methionine and threonine content [2]. However, the meat yield and chemical composition of various fish vary. Knowledge of these differences plays an important role in the nutritional and economic preference of these species. In addition to flavor, odor, color and texture as essential attributes of fish quality, other factors including age, size, growth rate, species, seasonal changes, feeding and killing techniques are equally involved in achieving the quality requirements [3–8]. Aquaculture products, which have an important share among foods with the code "blue food", are the focus of many studies/researchers. These blue foods, which are among the most traded products in the world, are protected by measures taken to ensure the safety and sustainability of the global food system [9–12]. In the ever-expanding seafood-food market, detailed studies have focused on production efficiency and fillet quality, which are important. Physical, chemical and microbiological analyses to determine the quality of processed products are developing in this parallel. The expectation from these analyzes is to provide fast and accurate results in the relevant product in a short time. They are important and pioneering analyses that usually include microbial load, aroma, some chemical (pH, TVB-N and TBARS) and physical (aw, WHC, color and texture) analyses [13]. High water activity (aw), high pH and the presence of autolytic enzymes are important factors in the high sensitivity of seafood products. Total volatile basic nitrogen (TVB-N) is one of the most preferred chemical variables for determining the freshness of seafood products. SDS-PAGE method is the most widely applied protein electrophoresis method used in the separation of meat proteins and in some studies to determine the meat species in meat products and mixtures. This method is mostly used to determine the molecular weight of proteins, to control protein purity, to fractionate proteins, to examine the substructure of pure protein [14].

One of the major causes that diminishes fillet quality and shortens shelf life is oxidation. Lipid oxidation in meat and meat products is most commonly measured by Thiobarbituric acid reactive substances (TBARS) analysis. Although lipid peroxidation is the inspiration for a multitude of studies, latest findings claimed that protein oxidation is of equal importance, and is even triggered by the reactive lipid oxidation products [13,15–18].

Although the importance of diet, species and season on fillet quality is known, the lack of studies to monitor the effect of age at slaughter on quality constitutes a deficiency in fish quality. Based on the above background explanation, in the present work we addressed for the first time the effect of slaughter age on fillet quality and mechanism of protein oxidation, as well as physico-chemical analysis as well as multiple assessment applications in terms of thermal stability, oxidation and profile of myofibrillar proteins (MPs).

2. Material and methods

Oncorhynchus mykiss obtained from Atatürk University Fisheries Faculty Inland Fish Research and Application Unit (toxic and disease certified manufacturer), were used as fish material.

A total of 40 male *Oncorhynchus mykiss* (rainbow trout) with an average age of 11–23 month were used in this study. These ages were preferred because they are the market size (11 months) and breeding stock candidate (23 months) time intervals for trout. At the determined temperatures: 12 ± 0.3 °C, O_2 level was adjusted by increasing the amount of water for survival comfort. Thanks to this setup, O_2 level was kept above 7.5–8 mg L^{-1} in all groups. At the end of the time when reached to the determined treatment age under controlled conditions, random samplings were made from the fish belonging to each age group [Group A: Average age of 11 month (A) and, Group B: Average age of 23 month (B)]. In order to determine the fillet quality fish were stunned via the fast neck-breaking technique was applied for the safety of the analyses and then decapitated and filleted without skin by hand under sterile conditions. For each age group, 40 fillets from 20 fish were prepared in 2 replicates, and the physico-chemical analysis and electrophoretic techniques were performed on randomly sampled fillets.

2.1. Physico-chemical analysis

In fillets divided into age groups, pH and chemical composition, the amount of crude ash, crude oil, crude protein, and dry matter were assessed according to Atamanalp [19]. In order to assess the water holding capacity (WHC), 5 g of each sample were centrifuged for 30 min at 4500 g/min and 10 °C. The supernatant was removed and after the pellet part was weighed, the WHC calculations were performed using the following formula [20].

$$\text{Water Holding Capacity } (\%) = [1 - (\text{pellet weight} / \text{first weight of the sample})] \times 100$$

Water activity (aw) measurements were made with an Aqualab water activity meter (Decagon Devices, model series 3) at 25 °C (±0.2 °C) [21]. Before the measurement, the device was calibrated using ready-made package (Decagon Devices, Inc. 2365 NE Hopkins Court Pulman WA 99163). Calibration solutions were defined by Aw values of 0.984 and 0.760, which are the closest to our reading values.

The intensity of the fillet color (a*, b* and L*) were obtained by using Minolta colorimeter (Minolta Co, CR-200, Osaka, Japan). The values were interpreted according to the recommendations of the Commission Internationale de l'Eclairage [a*; +a* = red, -a* = green, b*; +b* = yellow, -b* = blue color densities, L*; L* = 0 shows black; L* = 100, white (darkness/lightness)] [19,22].

Texture profile analysis (TPA) of the fillet was practiced using texture analyzer (CT3, Brookfield Engineering Laboratories, USA). Cylindrical samples of 20 mm diameter and 20 mm height were extracted from fillets with two press cylinders using a 50.8 mm probe (TA 25/1000, Brookfield Engineering Laboratories, USA) and were analyzed at room temperature. Transaction terms: the pre-test speed was set to 2 mm/s, the test speed and post-test speed were set to 1 mm/s, the time between the first and the second compression was 3 s, and the compression ratio was 50 %. The textural parameters (hardness, adhesiveness, resilience, cohesiveness, springiness, chewiness and gumminess) were assessed [23].

2.2. Determination of thermal stability, oxidation and profile of myofibrillar proteins

2.2.1. Thermal stability determination of miyofibrillar protein

Thermal changes of miyofibrillar protein were determined using Differential Scanning Calorimeter (DSC-60, Shimadzu Corp., Japan)]. Indium was used to calibrate the device for heat flux and temperature (Tm = 156.6 °C; ΔHm = 28.5 J/g). Approximately 10 mg of the sample was weighed into the aluminum sample cup and hermetically sealed. Heating was applied from 20 °C to 90 °C with a heating rate of 5 °C/min using an empty container placed in the sample device and sealed in the same way as a reference. The thermal changes of the analyzed samples were acquired on thermogram [13].

2.2.2. Protein oxidation

a. Muscle glycogen levels and lactic acid concentration

The glycogen level was estimated via the potassium hydroxide (KOH)/anthrone method, using a commercial kit (Abcam, Glycogen Assay Kit), within the first 6 h after following sampling, from the moment the rigor was formed, and was expressed as mg glycogen/g muscle [16]. The absorbance was read with UV–Vis spectrophotometer (Shımadzu) at 620 nm. Lactic acid concentration was assigned following the muscle stiffness began to develop (within the first 6 h after sampling) using the commercial kit (ChemBio, CB55560).

b. Myofibrillar protein (MPs) extraction and assessment of myofibrillar fragmentation index (MFI)

Samples homogenized with cold MFI buffer [40 mL of 0.02 M potassium phosphate buffer (pH 7.0)] containing 100 mM KCl, 1 mM EGTA, 1 mM $MgCl_2$ and 1 mM NaN_3, were centrifuged for 15 min at 1000×*g* and 4 °C. After the supernatant was removed and the pellet was resuspended in the same cold MFI buffer, the absorbance was measured using an UV–Vis spectrophotometer (Shımadzu) at 540 nm. The MFI was expressed by multiplying the absorbance by the dilution factor (200) [24,25].

c. Determination of sulfhydryl groups and disulfide bonds in myofibrillar protein

In order to assess the sulfhydryl groups and disulfide bonds content of MPs, the 5,5-dithiobis (2-nitrobenzoic acid) (DTNB) reaction was considered [26]. The MPs solution was mixed with 4.5 ml of 0.2 M Tris-HCl, 3 mM, ethylenediamine tetra acetic acid (EDTA), 1 % sodium dodecyl sulfate (SDS) buffer containing 8 ml of urea. Subsequently, 0.5 ml of buffer B (10 mM Tris-HCl, 10 mM DTNB, pH 8.0) was added over 4 ml of this mixture and incubated at 40 °C for 25 min. At the end of the incubation, the absorbance of the supernatant was measured at 412 nm, and the molecular absorbance coefficient of 13600 M^{-1} cm^{-1} was used to calculate the sulfhydryl content and the disulfide bonds content [16].

d. Carbonyl concentration

In the MPs samples the carbonyl content was determined by considering the 2,4-dithiophenylhydrazine (DNPH) method. For this purpose, MPs suspension was added to 10 mM DNPH solution and incubated for 1 h at room temperature. The obtained mixture was washed with 20 % trichloroacetic acid (TCA) and centrifuged at 10,000 rpm for 5 min. After the supernatant was discarded, the remaining pellet was resuspended in 3 ml of 6 M guanidine and incubated at 37 °C. After cooling, the absorbance was measured at 370 nm via spectrophotometer and the results were expressed as nM carbonyl/mg protein [27].

e. Determination of protein concentration

The amount of MPs was determined by the Biuret method [28]. For this purpose, 2 ml MP and oxide were taken as a sample, mixed with 3 ml of Biuret reagent and incubated at 37 °C for 15 min. Following the incubation, the mixture was centrifuged at 3500 rpm for 5 min and the absorbance of supernatant was read at 540 nm via UV–Vis spectrophotometer.

2.3. Determination of protein profile

Protein profile analysis (PPA) of the obtained homogenates was assessed using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) method [29]. 500 μl of commercial ready mix marker (Precision Plus Protein All Blue Prestained Protein Standard, Cat. No: 1610373) was used. Electrophoresis was performed in constant current mode (20 mA/gel) for approximately 90 min, gels were transferred in Oriole fluorescent gel stain for 2 h and gel imaging software system, Bio Rad Gel Doc XR was used to

visualize the gel.

2.3.1. Data analysis

In all trials, measurements were repeated three times and values were expressed as mean ± standard deviation (SD). Homogeneity tests were applied for all output using generalized linear models, differences were subjected to Student's t-test comparison tests (SPSS Ver. 22.0) and interpreted at $p < 0.05$.

3. Results and discussion

In seafood sector, although there are studies on fillet quality and shelf life, the effect of slaughter age on fillet quality and protein oxidation is still unclear. In this sense, our current research, in which detailed data is presented, provides important data for this gap as well as being a modeling study. However, there are no similar studies that can be compared with the research findings.

3.1. Interpretation of physico-chemical analysis

For fish products, pH serves as spoilage indicator. In our study, the pH value between the groups differentiated by age was found to be statistically important ($p < 0.05$). The pH value was determined as 6.79 ± 0.16 in the fillets belonging to group A and 6.46 ± 0.01 in group B (Table 1). The final pH value is a major marker for deciding about fillet quality. The higher pH in group A fillets could be due to distinctions in muscle fiber kinds or low muscle glycogen content. Similar results were previously reported by Si [30], which found that young animals may have higher final pH values than older animals because of a lack of glycogen.

The chemical ingredients of fillet are considered crucial reference marks for the evaluation of meat functionality. Fat, minerals and proteins are key components that show fillet quality, and moisture plays an essential role in maintaining the processing potential, quality and shelf life of seafood products. Age has a distinct effect on the fat and protein content of meat. In our study, When the effect of the slaughter age on the chemical composition of the fillets was examined, the ash, fat and moisture values were found to be statistically significant, but the changes in the protein content were not significant at the $p < 0.05$ level (Fig. 1). Chemical composition was significantly influenced by the age difference of the fish from the two experimental groups. Meats with high water-holding capacity (WHC) are preferred in the meat industry for economic reasons and because of the technology to be applied. The pH value has a significant effect on WHC, especially. Meats with low WHC and water-binding capacities can lead to undesirable changes such as high leakage water and cooking loss, as well as a decrease in cooking yield during processing, cooking, and storage [31,32].

It is thought that physical characteristics of fillets, including tenderness, juiciness and processing quality, may be sensitive to age, especially in the way of moisture conten [33]. One of the most important parameters for fish industry is WHC. An increased water content in the muscle has a negative impact on consumer demand by reducing mechanical strength and formation of extremely soft or tender fillets [34]. It is known that the net load of MPs, the membrane permeability of the muscle cell and its components, namely myofibrils, cytoskeletal connections, and the amount of extracellular space in the muscle are effective in fillet WHC values [35]. In our study, WHC values determined in rainbow trout fillets as 13.2 ± 0.89 and 14.8 ± 0.69 for group A and group B, respectively, were not found statistically significant at the $p < 0.05$ level (Table 1).

Aw is one of the main factors responsible for food stability and modulation of microbial response. Regarding the shelf life of fish Aw is a dominant parameter and has an important role on the growth of various microorganisms and spores [36]. In the present study, although relative changes in Aw activity were observed in fillets depending on slaughter age (group A = 0.9968 ± 0.003 and group B = 0.9965 ± 0.002), these differences were found to be statistically insignificant (Table 1).

Color is an essential tool for freshness and meat quality evaluation. This parameter is assigned by assessing redness (a*), yellowness (b*), lightness (L*) and is dependent on oxidation state - myoglobin concentration, which is affected by diet, breed, storage conditions and age [33]. When the effect of the slaughter age on the color quality of the fillets was examined, it was determined that it had an

Table 1
pH and color values of slaughter of different age on rainbow trout fillet.

	Slaughter age	Values
pH[a]	**A**	6.79 ± 0.16[a]
	B	6.46 ± 0.01[b]
WHC (%) [NS]	**A**	13.2 ± 0.89[a]
	B	14.8 ± 0.69[a]
Aw [NS]	**A**	0.9968 ± 0.003[a]
	B	0.9965 ± 0.002[a]
Colour parameters		
L[a]	**A**	48.99 ± 0.72[a]
	B	45.61 ± 0.13[b]
a[a]	**A**	−1.81 ± 0.85 [b]
	B	0.22 ± 0.68[a]
b[a]	**A**	9.17 ± 1.36 [b]
	B	15.03 ± 3.67[a]

Lowercase letters (a,b,c) show differences between groups determined in each parameters.
[a] $p < 0.05$, NS: not significant ($p > 0.05$) A: 11-month and B: 23-month.

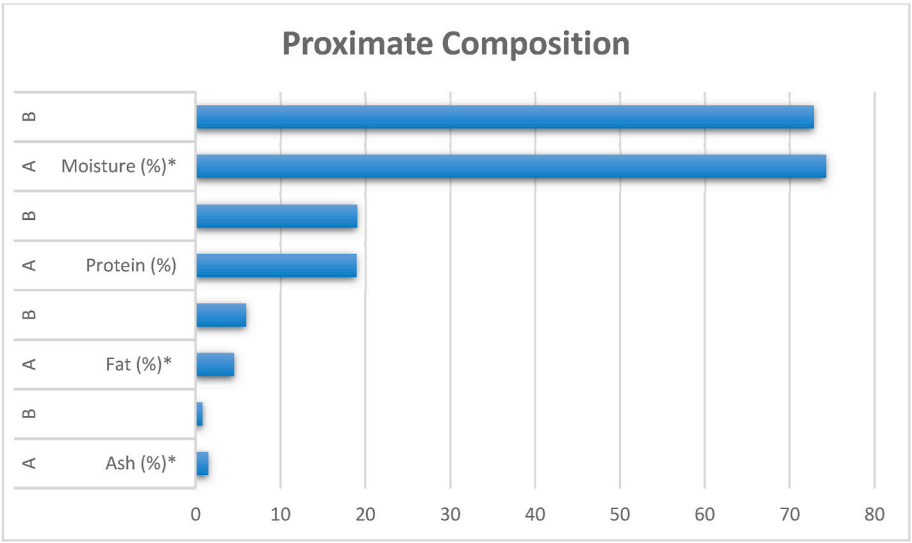

Fig. 1. Effect of slaughter age on fillets' chemical composition; *p < 0.05, A: 11-month and B: 23-month.

important effect on the L* and a* values (p < 0.05) (Table 1).

As seen in Table 1, group B fillets were defined by darker (lower L*) and redder (higher a*) scores than group A. A darker color is likely due to the effect of muscle fiber type with age and increasing myoglobin content. Regarding muscle type, there are reports that the light scattering properties of some muscles are increased by postmortem protein degradation, which promotes an increase in the L* value [30]. Colored components are often formed by protein oxidation and degradation, and a high degree of protein oxidation and degradation causes meat discoloration during aging [37,38]. In our study, a* value was found to be higher in group B. This is mainly associated with the presence of a natural reduction system in meat after slaughter and the presence of metmyoglobin. During aging, it is reduced to myoglobin by metmyoglobin reductase. However, metmyoglobin reductase activity gradually decreases with aging and the flesh color can change from red to brown [37,38].

Tissue parameters such as springiness, hardness, stickiness, chewiness and flexibility are generally used indicators for assessing the quality of fish products [39]. When the effect of slaughter age on texture of fillets was examined, it was seen that age had an important effect on all parameters (p < 0.05) (Table 2). An weaker connective tissue strength may be due to lower pH of tender fillets. It has been reported that higher pH of muscle has favorable effects for fillet tissue index, including firmness, chewiness, and springiness [40]. Other studies revealed that muscle fiber condition can have a significant positive correlation with fish stiffness [37,38]. Due to textural and structural changes in the postmortem period, the distribution and mobility of water in myofibrils may undergo changes. These affects the WHC and sensory properties of fillets [41]. In this process, changes in texture, flavor and color parameters are observed in fillets as a result of the effect of endogenous enzymes [42]. Our research findings support this situation in terms of pH and hardness. In our study, most relevant ascertainment is that the high hardness, chewing and stickiness values observed may be due to the higher WHC. Similar results were reported in carp fillets by Liu [43].

3.2. *Thermal stability, oxidation and profile interpretation of myofibrillar proteins*

The endothermic transition peaks (T*max*) and denatured enthalpy (△H) of the protein during heating are often used as reference marks of thermal stability. The two distinct endothermic peaks (T*max*1, T*max*2) assigned to the denatured temperature of MPs and the denatured enthalpy of myosin head for fillets of each age group and their corresponding enthalpies (△H1, △H2) are given in Table 3. The presented results show that T*max*2 for group A and B fillets increased by 72 % and 58.5 %, respectively, compared with MPs by slaughter age. This increase may be due to irreversible cross-bridge formation between myosin and actin [37,38]. In accordance with the previously presented results regarding the thermal stability of miyofibrillar protein, the energy required for the proteins

Table 2
Texture profile analysis of slaughter of different age on rainbow trout fillet.

Slaughter age	Texture paramaters						
	Hardness (N)[a]	Adhesiveness (mJ)[a]	Resilience[a]	Cohesiveness[a]	Springiness (mm)[a]	Gumminess (N)[a]	Chewiness (mJ)[a]
A	25.44 ± 1.15 [b]	0.55 ± 0.05 [b]	0.09 ± 0.01[a]	0.19 ± 0.02[a]	2.64 ± 0.09[a]	5.59 ± 0.90 [b]	10.90 ± 1.90 [b]
B	127.00 ± 6.92[a]	2.57 ± 0.28[a]	0.01 ± 0.00 [b]	0.06 ± 0.00 [b]	1.44 ± 0.24 [b]	7.61 ± 0.08[a]	15.38 ± 0.99[a]

Lowercase letters (a,b,c) show differences between groups determined temporally.
[a] p < 0.05, NS: not significant (p > 0.05), A: 11-month and B: 23-month.

Table 3
Change in the thermal stability of MPs of slaughter of different age on rainbow trout fillet.

	Denaturation temperature (°C)		Denaturation enthalpy (J/g)	
Slaughter age group	T_{max1}	T_{max2}	$_{\Delta}H_1$	$_{\Delta}H_2$
A	43.46 ± 1.94	74.42 ± 0.65	1.08 ± 0.33	0.46 ± 0.04
B	41.75 ± 0.13	65.76 ± 0.14	0.99 ± 0.20	0.07 ± 0.01

Different lowercase letters indicate significant difference in each column for each parameters ($P < 0.05$). Abbreviation: $_{\Delta}H$, denaturation enthalpy; SD: standard devition of the means ($n = 12$); T_{max}, peak temperature, A: 11-month and B: 23-month.

denaturation gradually increased in an age-related manner, and then decreased at a similar rate. These increases may indicate inhibition of proteolytic enzymes in denaturation, reduction in enthalpy and lower peaks at higher temperatures, denaturation of fillet proteins [13].

Heat-induced raised muscle protein-protein interactions and decreased water-holding capacity take place in 2 phases [44]. Among 30 and 50 °C coagulation of MPs occurs and the greatest reduce in WHC is obvious. From 55 to 90 °C, contraction of muscle fibers in the connective tissue network and increased interaction of the coagulated actomyosin system cause less water escape [45]. In our study, a significant and age-dependent decrease in Tmax2 values ($p < 0.05$), indicates a greater sensitivity of MPs in an age-related manner. Our findings are similar with those reported by Li [46] and Wang [37,38].

After the death of fish, the absence of oxygen transfer to the cells results in the body's reactions occurring under anaerobic conditions; at this stage, glycogen breaks down into lactic acid [16,47]. The degree of glycogen in muscle is the main marker of post-mortem pH due to lactic acid from anaerobic glycolysis [48]. In our study, glycogen levels were determined as 0.32 ± 0.04 and 0.37 ± 0.00 in A and B group fillets, respectively, and this difference was found to be insignificant at the $p < 0.05$ level (Table 4). An overall statistical analysis of the obtained results revealed that the glycolytic substrate increased and, as a result, the muscle pH decreased in group B fillets, which were defined by higher muscle glycogen stores. In addition, the lower glycogen level might be explained by a lower protein oxidation rate [49]. Following capture, even though fish have lost their vital functions post-capture, they are still exposed to post-mortem biochemical reactions with existing energy sources in the muscle tissue, such as ATP, glycogen, and other chemical compounds. Post-mortem changes in fish muscle can develop quickly or slowly depending on the fish species, size, and ambient temperature. In a living fish, the energy required for muscle contraction is provided by ATP produced during glycolysis. In this case, tissue ATP consumption and regeneration, contraction, and relaxation events continue constantly, whereas after post-mortem, with the cessation of blood circulation and oxygen supply in the tissue, the amount of ATP decreases rapidly, and contraction and relaxation events also continue in a limited manner during this decrease [16]. Our research has determined that the same killing technique used for each age group of the same fish species did not significantly affect the glycogen level and lactic acid amount, causing relative changes that the killing techniques applied did not have a significant impact. It is known that the killing technique has a significant effect on fish in endocrine responses and post-mortem biochemical processes (ATP breakdown and anaerobic glycolysis). It is thought that thrashing and struggling behaviors are effective in faster glycogen consumption. Decreases in muscle glycogen concentrations among age groups are due to the accumulation of lactic acid [47]. Increases in glycogen levels are thought to be caused by a low metabolic rate [50]. MFI is an index of protein degradation and meat tenderness [51]. It has been reported that the higher the MFI value, the worse the integrity of the internal structure of myofibrils [52]. In our study, the MFI showed that the totality of myofibrils and proteins clearly deteriorated with age, the spaces between myofibrils getting larger, and thus leading to severe fragmentation. The increment of MFI value may be due to the degradation of myonectin and accompanying actin in the I-band of the MPs sarcomere. It has also been shown that fragmentation of myofibrils may be as a result of integrity disruption near the Z line [53].

Myosin and actin are affluent in thiol groups, which will be turned into disulfide bonds after being attacked by intermolecular and intramolecular oxidation. A great number of sulfhydryl groups in the protein are located in the inner region of the molecule. Nevertheless, under the MDA oxidation system, the spatial structure of the protein changes, the structure expands and a large number of sulfhydryl groups are exposed, which can be lost following oxidation [37,38]. In our study, the age-related decrease of sulfhydryl content in MPs may be caused by the degradation of myosin, which leads to alterations in the spatial structure of the protein and causes the exposure and oxidation of embedded sulfhydryl groups [53].

Mainly, the sulfur atoms' high reactivity to oxidation, the oxidative conversion of cysteine to disulfide bonds and cysteine oxyaic

Table 4
Biochemical values of slaughter of different age on rainbow trout fillet.

Parameters	Slaughter age group	
	A	B
Glycogen level [NS]	0.32 ± 0.04[a]	0.37 ± 0.00[a]
Lactic acid (mmol/g protein) [NS]	0.42 ± 0.03[a]	0.53 ± 0.02[a]
MFI[a]	27.93 ± 0.11 [b]	42.80 ± 16.07[a]
S–S[a]	0.03 ± 0.005[a]	0.01 ± 0.002 [b]
S–H (μmoL/mg protein)[a]	0.16 ± 0.002[a]	0.11 ± 0.03 [b]
Carbonyl (mmol/mg)[NS]	0.71 ± 0.14[a]	0.92 ± 0.016[a]

Lowercase letters (a,b,c) show differences between groups determined temporally.
[a] $p < 0.05$, NS: Not significant ($p > 0.05$), A: 11-month and B: 23-month.

are effective in this reduction [54,55]. Carbonyl compounds are the main products of protein oxidation during meat processing [56]. As shown in Table 4, the carbonyl content of MPs increased remarkably in an age related-manner, but statistically insignificant ($p < 0.05$). The augmentation in carbonyl content is probably caused by carboxylation of the side chains of certain amino acids in myofibrils [55]. Increasing the carbon content and decreasing the sulfhydryl content can cause protein crosslinks that affect the constitution and spatial adjustment of MPs and reduce WHC [54]. The WHC results reported in our study are compatible with the previous reports. Thus, as a result of the MPs oxidation, validated by the increase of carbonyl content and the decrease of sulfhydryl content, the protein configuration and polarity change, affecting the water recombination of MPs.

3.3. Determination of protein profile

Oxidation can cause structural changes, including the structure of covalent bonds and the degradation of high molecular weight proteins to light molecular weight proteins. For this reason, SDS-PAGE is appreciated as a powerful technique for the assessment of the proteins oxidation extent [57]. β-mercaptoethanol, one of the components of this electrophoretic method, is a reducing agent that can break the disulfide bonds in SDS-PAGE [37,38].

In our study, examining the influence of slaughter age on rainbow trout quality fillets, the conventional model for MPs consisting of two main bands is clearly seen (Fig. 2). One of these bands is thought to be actin with a weight of about 42 kDa. Actin bands appear to be more intense than other bands, which is highly correlated with protein oxidation. However, the low-intensity bands in the obtained SDS-PAGE image are estimated to be protein chains produced by degradation of major histocompatibility complex (MHC). While dense band images indicate heavy molecular chain, protein cross-linking and protein accumulation, the appearance of light molecule chain can be interpreted as oxidation of the protein, which leads to degradation of the heavy molecular weight proteins [58].

Considering the research findings and literature to date, it has been noticed that the effect of harvest age on the quality of aquatic products has not been fully evaluated with a holistic approach, nor has any strategy been developed regarding this situation. Therefore, it is thought that the current study data will consider this parameter an important quality element in the processing of aquatic products and the food industry in the production of unprocessed, processed, semi-processed, and advanced processed products.

4. Conclusion

The study the influence of the slaughter age on the quality of the fillet. The authors identified undesirable changes in fillet properties such as chemical composition, color, and texture. Authors also reported alterations of pH, water holding capacity, oxidation, and thermal stabilization were related to slaughter age. Our results show that age at slaughter can affect important physical and sensory aspects of rainbow trout fillet quality. Overall, the findings indicated that increasing age has an impact on fillet quality serving the consumer market. In the collected findings, it was found that the myofibrils and proteins clearly deteriorated with age and slaughter age caused texture changes. In addition, the brightness of the fillets negatively affected with slaughter age.

Physical, chemical and microbiological changes in seafood after death such as rigor mortis, and enzymatic changes have a very important effect on fillet quality. In the postmortem period, the distribution and mobility of water in myofibrils may change due to textural and structural changes. This affects the water holding capacity and sensory attributes of fillets. Changes in texture, flavor and color parameters are observed in fillets as a result of the effect of endogenous enzymes. Nevertheless, the effect of slaughter age on protein oxidation and thermal stability, especially in fish, and their influence on fillet quality, should be further clarified.

Our study covers important gaps regarding the influence of the slaughter age on the quality of the fillet. Undesirable changes of fillet properties such as chemical composition, color and texture were noticed, which can lead to a diminution in consumer demands. In addition, other important alterations of pH, water holding capacity, oxidation and thermal stabilization were related to slaughter age. The findings obtained with the present research constitute a modeling study and created a useful data library for the elucidation of the mechanism by which the slaughter age can influence the quality of the fillet. Besides, these results offer a strong foundation for further advanced research of slaughter age in terms of lipid oxidation and microbial spoilage in the food industries.

Data availability statement

No new data associated with the manuscript.

CRediT authorship contribution statement

Ayşe Kara: Investigation, Formal analysis, Data curation. **Ahmet Akkose:** Investigation, Formal analysis. **Sevda Urçar Gelen:** Investigation, Formal analysis. **Arzu Uçar:** Investigation, Formal analysis. **Veysel Parlak:** Investigation. **Esat Mahmut Kocaman:** Investigation, Formal analysis. **Muhammed Atamanalp:** Writing – review & editing, Investigation, Investigation, Funding acquisition. **Nicoleta Anca Şuţan:** Writing – review & editing. **Ghadeer M. Albadrani:** Investigation, Funding acquisition. **Muath Q. Al-Ghadi:** Investigation, Funding acquisition. **Mohamed M. Abdel-Daim:** Writing – original draft, Resources, Project administration, Methodology, Investigation, Formal analysis, Conceptualization. **Gonca Alak:** Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to

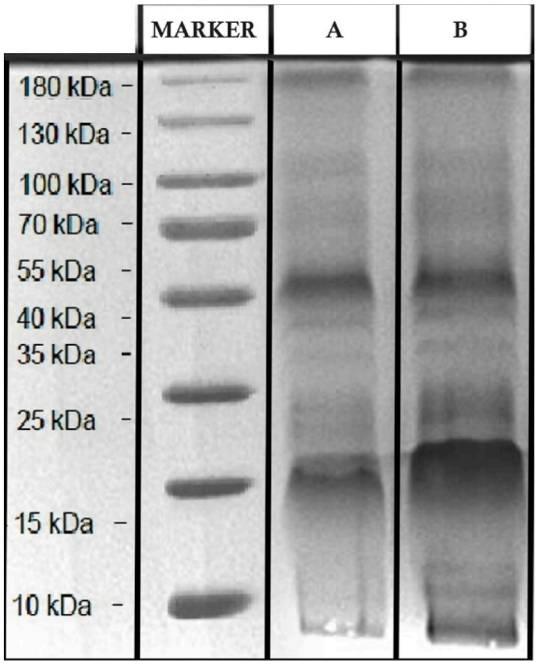

Fig. 2. SDS-PAGE image of miyofibrillar protein of slaughter age on rainbow trout fillets A: 11-month B: 23-month and Marker.

influence the work reported in this paper.

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Appendix A. Supplementary data

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