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7 Lipid damage in farmed rainbow trout (*Oncorhynchus*
8 *mykiss*) after slaughtering and chilling storage
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SUMMARY

Flow ice system including ozone (OFI condition) was tested for slaughtering and storage (up to 16 days) of farmed rainbow trout (*Oncorhynchus mykiss*). Lipid damage analyses were carried out and compared to sensory acceptance and instrumental colour changes. Comparison to individuals processed under flow ice system in the absence of ozone (FI condition) was undertaken. Rainbow trout slaughtered and chilled under FI and OFI conditions showed a low lipid damage development, according to lipid oxidation and hydrolysis events and lipid composition (polyunsaturated fatty acids, phospholipids and endogenous antioxidants) changes. Additionally, both icing conditions led to large good quality and shelf-life times and to absence of changes for colour properties. It is concluded that flow ice as such, or including the ozone presence, can be considered accurate strategies to be employed as slaughtering and storage systems during the commercialisation of the actual farmed species. The ozone presence has shown some profitable effects as leading to an extended shelf life time by quality retention of several sensory parameters; in contrast, some negligible negative effects could be assessed on the secondary and tertiary lipid oxidation development. However, oxidation values reached by individuals kept under OFI conditions can not be considered specially high.

Key Words: Rainbow trout, farming, slaughtering, chilling, flow ice, ozone, rancidity

Running Title: Lipid damage in iced farmed rainbow trout

1. INTRODUCTION

Fish species have attracted a great attention from consumers as a source of important constituents for the human diet [1, 2]. Thus, the fish lipid fraction is now the subject of a great deal of attention due to its high content on ω -3 polyunsaturated fatty acids (PUFA), according to their positive role in preventing certain human diseases [3]. However, from a technological point of view, a great number of studies have proved the incidence of PUFA oxidation in fish quality loss. As a result of enzymatic and non-enzymatic rancidity development, lipid oxidation compounds have shown to facilitate the off-flavour and odour formation and essential nutrient losses [4, 5].

Recent research accounts for advanced chilling strategies. One such technologies is flow ice (FI), which when employed in the place of traditional flake ice has shown many advantages such as a lower temperature, faster cooling, lower physical damage to the product and better heat exchange power. As a result, the application of this chilling strategy has led to an important inhibition of autolysis development, microbiological activity and lipid oxidation in different kinds of marine products [6-8].

Ozone is a powerful antimicrobial agent that is suitable for application in food in the gaseous and aqueous states leading to significant increases in sensory quality and shelf-life of fish [9]. Molecular ozone or its decomposition products inactivate microorganisms rapidly by reacting with intracellular enzymes, nucleic material and other components. In spite of its advantages as a food additive, the pro-oxidant behaviour of ozone on fish food constituents may denote a considerable drawback. Thus, a detrimental effect on phospholipid classes, polyunsaturated fatty acids and membrane proteins has been shown to occur [10, 11].

1 Since some decades, fish technologists and the fish trade have specially been attracted
2 by aquaculture development as a source of fish products. Among cultivated fish,
3 rainbow trout (*Oncorhynchus mykiss*) deserves a great attention because of its
4 increasing production in West- and North-Europe, Chile, USA and Japan [12]. Previous
5 research on this fresh water species accounts for the chemical composition analysis [13,
6 14], the effect of biological and technological factors on nutritional properties [15] and
7 farming conditions [16], this including the employment of specific diets and their
8 effects on quality during further processing or storage [17]. Concerning its
9 commercialisation as a fresh product, traditional refrigeration systems have been
10 applied [18, 19], taking into account the effect of a previous gutting process [20].
11 Meanwhile, advanced technologies such as high pressure [21], vacuum packaging [22]
12 and modified atmospheres [23] have been applied to obtain fresh rainbow trout.
13 The present work focuses on the commercialisation of farmed rainbow trout as a fresh
14 product. For it, FI system was chosen and applied as slaughtering medium and as
15 chilling storage system. With a view to extend the shelf life, a combined refrigeration
16 system consisting of ozone and flow ice (OFI) was evaluated comparatively. In this
17 study, lipid damage analyses were carried out and compared to sensory acceptance and
18 instrumental colour changes.

2. MATERIALS AND METHODS

2.1. Refrigeration systems

FI was prepared using a FLO-ICE prototype (Kinarca S.A.U., Vigo, Spain). The composition of the FI binary mixture was 40% ice and 60% water, prepared from filtered seawater (salinity: 3.3%). The temperature of the FI mixture was -1.5°C.

When required, the injection of ozone in the FI mixture was accomplished with a prototype provided by Cosemar Ozono (Madrid, Spain), the redox potential being adjusted to 700 mV (0.20 mg ozone/ l). In this batch, the ozone concentration was constantly monitored by checking the redox potential in the liquid phase.

2.2. Starting fish, slaughtering, chilling storage and chemicals

Specimens (108 individuals) of rainbow trout (*Oncorhynchus mykiss*) (weight range: 0.23-0.33 kg; length range: 25-30 cm) were obtained from an aquaculture facility (Isidro de la Cal, La Coruña, Spain) and were sacrificed at the farm by immersion in either FI (54 individuals) or OFI (54 individuals). In both systems, fish were surrounded by FI or OFI at a 1:1 fish-to-ice ratio and transported during 2 h at 0°C to the laboratory. Then, the fish specimens were maintained in their corresponding icing medium and directly placed in an isothermal room at 0°C.

On the next day (day 1), nine specimens from each icing batch were taken for analysis. Specimens from each icing condition were divided into three groups (three individuals in each group) that were studied separately (n = 3). Once fish specimens had been subjected to sensory and instrumental colour analyses, the white muscle was separated and employed for lipid quality assessment, as expressed below. Fish sampling was then

continued at days 3, 6, 9, 13 and 16 of refrigerated storage, according to the same sampling design (n = 3).

All solvents and chemical reagents used in the experiments were reagent grade (Merck, Darmstadt, Germany).

2.3. Sensory analysis

Sensory analysis was conducted by a sensory panel consisting of five experienced judges, according to guidelines concerning fresh and refrigerated fish (Table 1) [24].

Four categories were ranked: highest quality (E), good quality (A), fair quality (B) and unacceptable quality (C). The panellists included in this study had been involved in sensory analysis of different fish species during ten years. Previously to the present experiment, the panellists were specially trained with chilled rainbow trout.

Sensory assessment of the fish included the examination of the following parameters: skin, eyes, external odour, gills and consistency. At each sampling time, the fish specimens were presented to panellists and were scored individually. The panel members shared samples tested.

2.4. Instrumental colour analysis

Individual fishes were filleted by hand prior to instrumental colour analysis (CIE 1976 L*, a*, b*), that was performed by employing a tristimulus Hunter Labscan 2.0/45 colorimeter. Measurements were made directly on the rainbow trout fillets. For each sample analysis, colour scores were obtained as mean values of four measurements obtained by rotating the measuring head 90° between duplicate measurements per position.

2.5. Proximate analyses

Moisture content was determined by the difference between the weight of fresh homogenised muscle (1-2 g) and the weight recorded after 24 h at 105 °C. Results were expressed as g water/ 100 g muscle.

Lipids were extracted by the Bligh and Dyer [25] method. Quantification results were expressed as g lipid/ 100 g muscle.

NaCl content in fish muscle was determined by a modification of the Volhard method, which included boiling in concentrated (60%) HNO₃, neutralisation of NaCl meq with excess of 0.1 N AgNO₃, and final determination of the excess of AgNO₃ meq by reverse titration with 0.1 N NH₄SCN [26]. Results were expressed as g NaCl/ 100 g muscle.

2.6. Lipid composition analyses

Total phospholipids (PL) were determined by measuring the organic phosphorus on total lipid extracts, according to the Raheja et al. [27] method based on the formation of a complex with ammonium molybdate. The results were expressed as g PL/ 100 g lipids.

Lipid extracts were converted into fatty acid methyl esters (FAME) by employing acetyl chloride and then analysed by gas chromatography according to previous procedure [28]. FAME were analysed by means of a Perkin-Elmer 8700 chromatograph employing a fused silica capillary column SP-2330 (0.25 mm i.d. x 30 m, Supelco Inc., Bellefonte, PA, USA). Nitrogen at 10 psi as carrier gas and flame ionisation detector (FID) at 250°C were used. Peaks corresponding to fatty acids were identified by comparison of their retention times with standard mixtures (Larodan, Qualmix Fish; Supelco, FAME Mix). Peak areas were automatically integrated, 19:0 fatty acid being used as internal standard for quantitative analysis. The concentration of each fatty acid

was calculated as g/ 100 g total FAME. The polyene index (PI) was calculated as the following fatty acid ratio: C20:5 + C22:6/ C16:0.

Astaxanthin content was measured according to Sheehan et al. [29]. For it, fish muscle was extracted with acetone. The combined extracts were carried out to dryness under nitrogen flux and dissolved in the mobile phase, which consisted of 20% ethyl acetate and 80% methanol-water (9:1). HPLC separation of the samples was carried out on a Nucleosil 5 C18 (25 cm x 4 cm i.d.) reverse phase column, being detection carried out at 470 nm. Absence of 9Z- and 13Z-isomers was confirmed; only E-isomers were detected in the present rainbow trout samples. Results were expressed as mg all-E-astaxanthin (AX)/ kg fish muscle.

Tocopherols were analysed according to the method of Cabrini et al. [30]. For it, lipophilic antioxidants were extracted from the muscle with heptane, carried out to dryness under nitrogen flux, dissolved in isopropanol and injected in the HPLC analysis. An ultrasphere ODS column (15 cm x 0.46 cm i.d.) was employed, by applying a gradient from 0 to 50% of isopropanol. Flow rate was 1.5 ml/ min. Detection was achieved at 280 nm. Alpha, gamma and delta isomers were detected in the subject rainbow trout samples, and their content was expressed as mg/ kg fish muscle.

2.7. Lipid damage assessment

Free fatty acid (FFA) content was determined by the Lowry and Tinsley [31] method based on complex formation with cupric acetate-pyridine. Results were expressed as g FFA/ 100 g lipids.

The peroxide value (PV) was determined according to the ferric thiocyanate method [32]. Results were expressed as meq active oxygen/ kg lipids.

The thiobarbituric acid index (TBA-i) was determined according to Vyncke [33]. Results were expressed as mg malondialdehyde/ kg fish muscle. Formation of fluorescent compounds was determined by measurements at 393/463 nm and 327/415 nm as previously described [34]. The relative fluorescence (RF) was calculated as follows: $RF = F/F_{st}$, where F is the fluorescence measured at each excitation/emission maximum, and F_{st} is the fluorescence intensity of a quinine sulphate solution (1 µg/ ml in 0.05 M H₂SO₄) at the corresponding wavelength. The fluorescence ratio (FR) was calculated as the ratio between the two RF values: $FR = RF_{393/463} \text{ nm} / RF_{327/415} \text{ nm}$. The FR value was determined in the lipid extract.

2.8. Statistical analyses

Data corresponding to the two icing conditions were subjected to one-way analysis of variance to assess significant ($p < 0.05$) differences among treatments [35]; the effect of the chilled storage time was also analysed ($p < 0.05$). The SPSS 11.5 software for Windows (SPSS Inc., Chicago, Il, USA) was also used to explore the statistical significance of the results obtained, including multivariate contrasts and multiple comparisons by the Scheffé and Tuckey tests; a confidence interval at the 95% level was used in all cases.

3. RESULTS AND DISCUSSION

3.1. Proximate composition

The moisture and lipid contents ranged from 73.5-75.8 and 0.75-1.15 g/ 100 g muscle, respectively. Values for these constituents did not provide significant differences

($p>0.05$) as a result of the icing conditions nor as a result of the icing time. Lipid content was found similar to the one reported for the same species obtained from wild conditions [13], but lower when compared to previous research on farmed fish individuals [13, 19, 20, 36]. According to a known inverse ratio between moisture and lipid matter [1], a higher moisture content was obtained in the present study than in previous research for farmed rainbow trout [19, 20, 36].

An increasing ($p<0.05$) NaCl content was observed throughout the experiment for both icing conditions (Figure 1). This increase can be explained as a result of the NaCl presence in the refrigeration systems employed and agrees to previous research concerning wild species stored under flow ice conditions [7, 8]. Throughout most of the present experiment, no differences ($p>0.05$) in NaCl content could be outlined between individuals slaughtered and kept under both icing conditions; the exception was found at day 13 when a slightly higher ($p<0.05$) content was observed for fish treated under FI condition. In spite of this general NaCl content increase with time, present NaCl levels attained are found much more smaller than those obtained after refrigeration in seawater [37].

3.2. Lipid composition analyses

PUFA breakdown was measured by following the PI of lipids in the white muscle (Table 2). This parameter showed no significant differences ($p>0.05$) as a result of the icing system, neither as a result of the icing time. Present results (PI range: 2.6-2.9) are found markedly higher than those reported for the same species in a previous study [20], this being attributive to the effect of the diet provided [13, 19]. Previous research has shown that wild fresh water fish is constituted by a lower content of long chain polyunsaturated fatty acids than wild marine fish [38]. However, present PI results are

found to be in the same range than those obtained for marine species [28, 34], this being attributive again to the effect of the diet provided [13, 19].

PL classes have been described to be important components of cell membranes and be constituted by a high PUFA content [28, 38]. Accordingly, their content was measured in order to assess possible PUFA damage. Results obtained concerning the total PL content (Table 2) were included in the range 30-39 g/ 100 g lipids, and did not show significant differences ($p>0.05$) as a result of the icing system employed, neither as a result of the icing time. PL proportion in lipids has shown an inverse ratio with total lipid content; in this sense, present PL contents agreed to low-lipid-content species [28, 38], and are found markedly higher than those reported for the same farmed species where fish individuals showed a higher lipid content [20].

Tocopherol isomers and carotenoids like astaxanthin (AX) are known as relevant endogenous antioxidants that can act as scavengers of free radicals, so that protection against lipid oxidation would be favoured and accordingly, PUFA content and composition maintained [17, 39].

In the present experiment, the AX content and the presence of different tocopherol isomers was analysed (Table 3). Values for AX, α -tocopherol, γ -tocopherol and δ -tocopherol did not provide significant differences ($p>0.05$) as a result of the icing system employed, neither as a result of the icing time. Content variations among samples can be explained as a result of fish-to-fish differences.

Previous research on rainbow trout has shown that when stored under refrigeration conditions, AX [22] and α -tocopherol [19] were partially lost as fish damage increased by storage time increasing. Since a content decreasing tendency was not obtained for any of the antioxidant molecules checked in the present study, it is concluded that FI

and OFI systems provide satisfactory slaughtering and chilling conditions for the lipid oxidation stability.

3.3. Lipid hydrolysis

FFA content in rainbow trout muscle showed a marked increase ($p<0.05$) for both icing conditions after 3 days (Figure 2). Then, a period of no significant changes ($p>0.05$) could be observed. Higher mean values were obtained at all sampling times for individuals kept under OFI conditions than in their counterparts under FI, this difference being significant ($p<0.05$) at day 9. In a previous work [40], a lower FFA formation was observed in turbot chilled under OFI condition when compared to its counterpart under FI treatment; however, such result was obtained after 35 days of chilled storage, and a differential FI/ OFI slaughtering process was not encountered.

FFA formation has been reported to be produced during a first stage of the chilling process (up to days 6-9, approximately) as a result of endogenous enzyme (namely, lipases and phospholipases) activity [41, 42]. Later on, microbial activity should be important, so that FFA formation should mostly be produced as a result of bacterial activity. Present results on FFA formation show values below 1 % in all cases, which can be considered lower than those obtained for most marine lean fish species when kept under chilling conditions [6, 34]. In addition, a higher FFA formation during chilling storage could be observed for the same farmed species [20]. Accordingly, a low lipid hydrolytic activity is concluded to occur after slaughtering and chilling storage of the present fresh water species under both FI and OFI conditions.

While the formation of FFA itself does not lead to nutritional losses, its assessment is deemed important when considering the development of rancidity. Thus, a pro-oxidant effect of FFA on lipid matter has been proposed and explained on the basis of a

1 catalytic effect of the carboxyl group on the formation of free radicals by the
2 decomposition of hydroperoxides [43]. In this sense, Han and Liston [44] provided a
3 marked support for a straight correlation between lipid peroxidation and phospholipid
4 hydrolysis in frozen (-10°C) rainbow trout.

6 **3.4. Lipid oxidation**

7 Lipid oxidation was measured by the peroxide development (primary oxidation), the
8 thiobarbituric acid reactive substances (TBARS) formation (secondary oxidation) and
9 the assessment of interaction compounds produced between primary and secondary
10 lipid oxidation compounds and nucleophilic compounds (namely, protein-like
11 molecules) (tertiary oxidation).

12 Peroxide formation was very low in all cases (PV range: 1.0-4.0) (Table 2), being
13 similar to the results obtained by applying FI to a lean fish species [6] and lower than
14 the peroxide development observed in a lean fish species chilled under flake icing [34].
15 In the present work, a clear tendency with time could not be observed for individuals
16 treated under both icing conditions ($p>0.05$); only some small differences between icing
17 conditions could be assessed for the PV, these leading to no conclusions concerning the
18 ozone presence effect, according to previous research [40]. Low scores obtained in the
19 present experiment for the peroxide formation agree to the retention of AX content,
20 according to the scavenger role of this carotenoid compound in the very early stages of
21 lipid oxidation [17].

22 More important than primary was secondary lipid oxidation development (Table 2).
23 Thus, TBARS values were included in the range 0.15-0.60, which can be considered
24 similar to scores found for a medium-fat fish [8], but fast lower than those reported for a
25 fatty fish [7], all stored under FI conditions. A definite effect of icing time could not be

assessed ($p>0.05$) for both icing systems in the present work. Comparison between both slaughtering and storage conditions showed higher mean values for individuals under OFI condition, although significant differences could only be assessed at days 1 and 3. A slight pro-oxidant effect of ozone presence could be concluded, which does not agree to previous work [40]. In such study, a lower TBARS formation was observed in turbot chilled under OFI condition when compared to its counterpart under FI treatment; however, such result was obtained in the 28-35 day period of chilled storage, and a differential FI/ OFI slaughtering process was not encountered.

The fluorescence formation (FR parameter) did not provide differences ($p>0.05$) between both icing conditions (Table 2). However, higher mean values for individuals slaughtered and kept under OFI condition were obtained in the 9-16 days period, this according to the above mentioned TBARS formation. An increasing ($p<0.05$) FR tendency with icing time could be depicted for both storage systems, that can be explained as a result of an increasing lipid damage. However, FR values obtained for both kinds of fish individuals can be considered notably low, according to previous research on chilling storage of wild species kept under traditional flake ice [7, 8, 34].

3.5. Sensory attributes

Progressive decreases in scores were observed in samples from both treatments throughout the experiment (Table 4). However, a good quality (E and A marks) was maintained for all kinds of individual fishes up to day 6, which can be considered a profitable commercial result. Differences with previous results on the same chilled species [18, 20] could be explained as a result of biological factors of individuals employed in the different studies such as size and lipid content [15, 38].

1 Fish samples slaughtered and chilled under FI conditions showed a shelf life time of 13
2 days, while their counterpart individuals treated with the OFI system were still
3 acceptable at the end of the experiment, although all parameters were mark B. In a
4 previous research [18], the same species was found to be unacceptable at day 6 when
5 kept under traditional ice. Throughout the chilled storage some differences could be
6 assessed between individual fishes corresponding to both icing conditions; thus, a better
7 score was obtained for individuals treated with OFI system for external odour (days 13
8 and 16), skin (days 6, 9 and 13) and eyes (day 13). As a result, a profitable effect of
9 ozone presence can be concluded from sensory evaluation, this agreeing to a previous
10 research carried out on a wild fatty fish species [7]. Such valuable effect can be
11 attributed to its strong antimicrobial power [9].

12 Among the different chemical lipid damage parameters studied in the present
13 experiment, secondary lipid oxidation compounds are known to be the most closely
14 related to the formation of oxidised flavours [45]. In the present experiment, a marked
15 increase in TBARS formation was not observed, this agreeing to the lack of rancid
16 odour detection during the sensory evaluation.

18 **3.6. Colour analysis**

19 Colour plays an important role in the appearance, presentation and acceptability of fish
20 food. In the present study, sensory evaluation was complemented with instrumental
21 colour analysis (Table 5).

22 Values for lightness (L^*) parameter did not provide significant differences ($p>0.05$) as a
23 result of the icing time, and were included in the range 54.5-64.4. Individuals treated
24 under OFI condition showed some higher values (days 9 and 13) than their counterparts
25 treated with FI system; however, this tendency was not maintained at the end of the

experiment. Concerning redness (a^*) and yellowness (b^*) parameters, differences could not be obtained ($p>0.05$) as a result of icing time, neither by comparing both icing conditions. These results agree to previously mentioned data showing no changes for the AX content (Table 3).

Previous research related to the refrigerated storage (4°C) of rainbow trout fillets packed under vacuum did not provide differences up to 15 days of storage for colour parameters (L^* , a^* and b^*) [22]. However, colour changes were observed for this species when previously treated under hydrostatic high pressure and then refrigerated (4°C) [21] or during the frozen storage (-18°C) [46].

Redness (a^*) loss has been proposed as a way of following haemoglobin-mediated lipid oxidation in fish [47], showing an inverse relationship with secondary lipid oxidation (TBARS) development [47, 48]. In the present experiment, none of both parameters (TBARS formation and a^*) provided a definite tendency with icing time.

Concerning yellowness (b^*) development, an important relationship with the formation of polymerised Schiff bases and fluorescent compounds (tertiary lipid oxidation compounds) has been observed [6, 49]. However, the increasing FR values obtained throughout the present experiment were not followed by a b^* parameter increase.

4. CONCLUSIONS

Farmed rainbow trout slaughtered and chilled under FI and OFI conditions has shown a lipid composition relatively stable, so that a low lipid damage development and a marked sensory quality retention were obtained. Thus, a low lipid hydrolysis and oxidation development could be observed, that was followed by the absence of lipid

composition (PUFA, PL and endogenous antioxidants) and instrumental colour changes. Concerning the sensory acceptance, remarkable good quality and shelf-life times were obtained. According to present demands on the quality of fresh water farmed fish species, it is concluded that flow ice as such, or including the ozone presence, can be considered accurate strategies to be employed as slaughtering and chilling systems for providing good quality products. The ozone presence has shown some profitable effects as leading to an extended shelf life time by quality retention of several sensory parameters. In contrast, some negligible negative effects could be observed on the secondary and tertiary lipid oxidation development. However, oxidation values reached by individuals kept under OFI conditions can not be considered specially high.

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FIGURE LEGENDS

Figure 1: Comparative NaCl assessment in farmed rainbow trout muscle after slaughtering and chilled storage in flow ice (FI) and ozonised flow ice (OFI)

* Mean values of three ($n = 3$) independent determinations. Standard deviations are indicated by bars.

Figure 2: Comparative free fatty acid (FFA) formation in farmed rainbow trout muscle after slaughtering and chilled storage in flow ice (FI) and ozonised flow ice (OFI)

* Mean values of three ($n = 3$) independent determinations. Standard deviations are indicated by bars.

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Figure 1

Figure 2

TABLE 1

Scale employed for evaluating the freshness degree of farmed rainbow trout

Attribute	Highest quality (E)	Good quality (A)	Fair quality
Skin	Very intense pigmentation; transparent mucus	Milky mucus; insignificant pigmentation losses	Slightly greyish pigmentation with
Eyes	Convex; transparent cornea; bright and black pupil	Convex and slightly sunken; slightly opalescent cornea; black and cloudy	Flat; opalescent opaque pu

		pupil	
External odour	Sharply seaweedy and shellfish smell	Weakly seaweedy and shellfish smell	Incipiently putrid
Gills	Brightly red; without odour; lamina perfectly separated	Rose coloured; without odour; lamina adhered in groups	Slightly pale; in fishy odour; lamina adhered in groups
Consistency	Presence or partial disappearance of rigor mortis symptoms	Firm and elastic; pressure signs disappear immediately and completely	Presence of mechanical signs; elasticity reduced

TABLE 2

Comparative lipid parameter assessment* in farmed rainbow trout after slaughtering and chilled storage in flow ice (FI) and ozonised flow ice (OFI)**

Icing Time (days)	Polyene Index		Phospholipids (g/ 100g lipids)		Peroxide Value (meq active oxygen/ kg lipids)		Thiobarbituric acid index (mg malondialdehyde/ kg muscle)		Fluorescence Ratio	
	FI	OFI	FI	OFI	FI	OFI	FI	OFI	FI	OFI
1	2.7 (0.3)	2.6 (0.3)	35.2 (2.2)	34.6 (3.6)	2.7 a (0.7)	3.4 b (0.1)	0.22 (0.07)	0.36 (0.09)	0.03 (0.01)	0.05 (0.02)
3	2.9 (0.2)	2.8 (0.2)	34.6 (5.3)	36.2 (5.0)	1.2 (0.1)	1.3 (0.1)	0.34 a (0.08)	0.55 b (0.07)	0.07 (0.02)	0.05 (0.01)
6	2.9 (0.2)	2.8 (0.2)	32.8 (2.3)	32.9 (2.2)	2.6 (0.2)	2.6 (0.9)	0.41 (0.12)	0.51 (0.04)	0.07 (0.02)	0.06 (0.01)
9	2.8 (0.3)	2.9 (0.4)	33.7 (4.0)	32.3 (3.6)	3.7 b (0.2)	2.4 a (0.6)	0.26 (0.08)	0.34 (0.12)	0.06 (0.02)	0.11 (0.04)
13	2.6 (0.3)	2.8 (0.2)	36.1 (3.0)	33.9 (3.7)	3.5 (1.1)	3.3 (0.7)	0.27 (0.06)	0.36 (0.12)	0.11 (0.04)	0.17 (0.03)
16	2.7 (0.2)	2.6 (0.3)	37.1 (2.2)	33.9 (2.8)	1.3 a (0.1)	2.0 b (0.4)	0.30 (0.03)	0.36 (0.08)	0.20 (0.04)	0.27 (0.05)

* Average values of three (n = 3) independent determinations. Standard deviations are indicated in brackets.

** For each parameter, average values followed by a different letter (a, b) denote significant differences ($p < 0.05$) between both icing conditions.

Comparative endogenous antioxidant assessment (mg/ kg fish muscle)* in farmed rainbow trout after slaughtering and chilled storage in flow ice (FI) and ozonised flow ice (OFI)**

* Average values of three (n = 3) independent determinations. Standard deviations are indicated in brackets.

TABLE 4[illegible]

6	A	E	E	E	E	E	A	A	A	A
9	B	A	A	A	A	A	B	B	A	A
13	B	A	B	A	B	A	B	B	A	A
16	B	B	B	B	C	B	B	B	B	B

* Freshness categories as expressed in Table 1.

TABLE 5

Comparative assessment of colour changes* in farmed rainbow trout after slaughtering and chilled storage in flow ice (FI) and ozonised flow ice (OFI)**

Icing Time (days)	Lightness (L*)		Redness (a*)		Yellowness (b*)	
	FI	OFI	FI	OFI	FI	OFI
1	58.3 (0.2)	56.9 (2.4)	23.3 (0.9)	21.3 (3.4)	30.4 (0.3)	28.9 (1.6)
3	57.7 (0.4)	58.7 (0.7)	24.0 (3.0)	25.3 (1.9)	31.2 (0.6)	31.2 (1.1)
6	60.4 (0.4)	62.2 (2.2)	22.7 (3.0)	23.9 (1.7)	31.7 (2.1)	30.5 (1.2)
9	57.6 a (1.3)	60.1 b (0.4)	26.4 (4.7)	19.4 (3.2)	31.2 (1.8)	29.9 (1.3)
13	55.6 a (1.3)	60.7 b (2.1)	24.6 (4.8)	24.2 (0.6)	31.6 (1.9)	30.2 (0.1)
16	59.0 (3.3)	57.5 (1.2)	22.9 (5.5)	22.4 (2.9)	29.4 (3.1)	29.1 (1.0)

* Average values of three (n = 3) independent determinations. Standard deviations are indicated in brackets.

** For each parameter, average values followed by a different letter (a, b) denote significant differences (p<0.05) between both icing conditions.