Lipid damage in farmed rainbow trout (*Oncorhynchus mykiss*) after slaughtering and chilled storage

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The 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 with the 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 largely good quality and shelf life times and to the absence of changes in colour properties. It is concluded that flow ice as such, or including the presence of ozone, can be considered as ideal strategy to be employed as slaughtering and storage system 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 observed on the secondary and tertiary lipid oxidation development. However, the oxidation values reached by individuals kept under OFI conditions cannot be considered as particularly high.

Keywords: Chilling / Farming / Flow ice / Ozone / Rainbow trout / Rancidity / Slaughtering

1 Introduction

Fish species have attracted great attention from consumers as a source of important constituents of 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 in *n*-3 polyunsaturated fatty acids (*n*-3 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 technology 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 to 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 rapidly inactivate microorganisms 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 (PL) classes, PUFA, and membrane proteins has been shown to occur [10, 11].

Since some decades, fish technologists and the fish trade have specially been attracted by aquaculture development as a source of fish products. Among cultivated fish, rainbow trout

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(*Oncorhynchus mykiss*) deserves great attention because of its increasing production in West- and North-Europe, Chile, USA and Japan [12]. Previous research on this freshwater species accounts for the chemical composition analysis [13, 14], the effect of biological and technological factors on nutritional properties [15] and farming conditions [16], including the employment of specific diets and their effects on quality during further processing or storage [17]. Concerning its commercialisation as a fresh product, traditional refrigeration systems have been applied [18, 19], taking into account the effect of a previous gutting process [20]. Meanwhile, advanced technologies such as high pressure [21], vacuum packaging [22] and modified atmospheres [23] have been applied to obtain fresh rainbow trout.

The present work focuses on the commercialisation of farmed rainbow trout as a fresh product. For this, the FI system was chosen and applied as slaughtering medium and as chilled storage system. With a view to extend the shelf life, a combined refrigeration system consisting of ozone and flow ice (OFI) was evaluated in comparison. In this study, lipid damage analyses were carried out and compared to sensory acceptance and 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 into the FI mixture was accomplished with a prototype provided by Cosemar Ozono (Madrid, Spain), with the redox potential 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, chilled 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 the fish specimens had been subjected to sensory and instrumental colour analyses, the white muscle was separated and employed for lipid quality assessment, as described below. Fish sampling was then continued on 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 of reagent grade (Merck, Darmstadt, Germany).

2.3 Sensory analysis

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

Table 1. Scale employed for evaluating the freshness degree of farmed rainbow trout.

Attribute	Highest quality (E)	Good quality (A)	Fair quality (B)	Unacceptable (C)
Skin	Very intense pigmentation; transparent mucus	Milky mucus; insignificant pigmentation losses	Slightly greyish mucus; pigmentation without shine	Widely opaque mucus; important pigmentation losses
Eyes	Convex; transparent cornea; bright and black pupil	Convex and slightly sunken; slightly opalescent cornea; black and cloudy pupil	Flat; opalescent cornea; opaque pupil	Concave and milky cornea; internal organs blurred
External odour	Sharply seaweedy and shellfish smell	Weakly seaweedy and shellfish smell	Incipiently putrid or rancid	Putrid or rancid
Gills	Brightly red; without odour; lamina perfectly separated	Rose coloured; without odour; lamina adhered in groups	Slightly pale; incipient fishy odour; lamina adhered in groups	Grey-yellowish colour; intense ammonia odour; lamina totally adhered
Consistency	Presence or partial disappearance of rigor mortis symptoms	Firm and elastic; pressure signs disappear immediately and completely	Presence of mechanical signs; elasticity notably reduced	Important shape changes due to mechanical factors

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 10 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 the panellists and 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*), which 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.

The NaCl content in fish muscle was determined by a modification of the Volhard method, which included boiling in concentrated (60%) HNO_3, neutralisation of NaCl meq with excess of 0.1 N $AgNO_3$, and final determination of the excess of $AgNO_3$ meq by reverse titration with 0.1 N NH_4SCN [26]. Results were expressed as g NaCl/100 g muscle.

2.6 Lipid composition analyses

Total PL were determined by measuring the organic phosphorus in 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 a 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. × 30 m; Supelco, Bellefonte, PA, USA). Nitrogen at 10 psi as carrier gas and a 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, using the 19:0 fatty acid 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: (20:5 + 22:6)/16:0.

The astaxanthin (AX) content was measured according to Sheehan *et al.* [29]. For this, fish muscle was extracted with acetone. The combined extracts were dried 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 × 4 cm i.d.) reverse-phase column; detection was carried out at 470 nm. The absence of 9*Z*- and 13*Z*-isomers was confirmed; only *E*-isomers were detected in the present rainbow trout samples. Results were expressed as mg all-*E*-AX/kg fish muscle.

Tocopherols were analysed according to the method of Cabrini *et al.* [30]. For this, lipophilic antioxidants were extracted from the muscle with heptane, dried under nitrogen flux, dissolved in isopropanol and injected into the HPLC system. An ultrasphere ODS column (15 cm × 0.46 cm i.d.) was employed, by applying a gradient from 0 to 50% of isopropanol. The flow rate was 1.5 mL/min. Detection was achieved at 280 nm. α-, γ- and δ-isomers were detected in the subject rainbow trout samples, and their content was expressed as mg/kg fish muscle.

2.7 Lipid damage assessment

The 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 H_2SO_4) at the corresponding wavelength. The fluorescence ratio (FR) was calculated as the ratio between the two RF values: $FR = RF_{393/463\ nm}/RF_{327/415\ nm}$. 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 to 75.8 and 0.75 to 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. The lipid content was found to be 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 on farmed rainbow trout [19, 20, 36].

An increasing ($p < 0.05$) NaCl content was observed throughout the experiment for both icing conditions (Fig. 1). This increase can be explained as a result of the NaCl presence in the refrigeration systems employed, and agrees with previous research concerning wild species stored under FI 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, with the exception of day 13 when a slightly higher ($p < 0.05$) content was observed for fish treated under FI conditions. In spite of this general NaCl content increase with time, the present NaCl levels attained are found to be much smaller than those obtained after refrigeration in seawater [37].

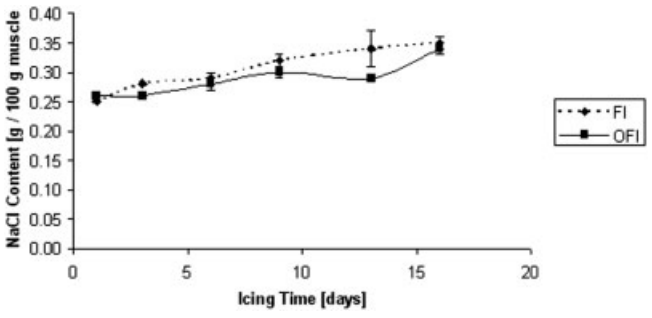

Figure 1. Comparative NaCl assessment in farmed rainbow trout muscle after slaughtering and chilled storage in FI and OFI. * Mean values of three ($n = 3$) independent determinations. Standard deviations are indicated by bars.

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, nor as a result of the icing time. The present results (PI range: 2.6–2.9) are found to be markedly higher than those reported for the same species in a previous study [20], due to the effect of the diet provided [13, 19]. Previous research has shown that wild freshwater fish has a lower content of long-chain PUFA than wild marine fish [38]. However, the present PI results are found to be in the same range as those obtained for marine species [28, 34], again due to the effect of the diet provided [13, 19].

PL classes have been described to be important components of cell membranes and to be characterised by a high PUFA content [28, 38]. Accordingly, their content was measured in order to assess possible PUFA damage. The results obtained concerning the total PL content (Table 2) ranged between 30 and 39 g/100 g lipids and did not show significant differences ($p > 0.05$) as a result of the icing system employed, nor as a result of the icing time. The PL proportion in lipids has shown an inverse ratio with total lipid content; in this sense, the present PL contents agreed with low-lipid-content species [28, 38] and were found to be markedly higher than those reported for the same farmed species where fish individuals showed a higher lipid content [20].

Tocopherol isomers and carotenoids like 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, nor 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 with increasing storage time. Since a tendency of decreasing content was not obtained for any of the antioxidant molecules checked in the present study, it is concluded that the FI and OFI systems provide satisfactory slaughtering and chilling conditions with regard to lipid oxidation stability.

3.3 Lipid hydrolysis

The FFA content in rainbow trout muscle showed a marked increase ($p < 0.05$) for both icing conditions after 3 days (Fig. 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

Table 2. Comparative lipid parameter assessment[§] in farmed rainbow trout after slaughtering and chilled storage in FI and OFI.[$]

Icing time [days]	PI		PL [g/100 g lipids]		PV [meq active oxygen/ kg lipids]		TBA-i [mg malondialdehyde/kg muscle]		FR	
	FI	OFI	FI	OFI	FI	OFI	FI	OFI	FI	OFI
1	2.7	2.6	35.2	34.6	2.7[a]	3.4[b]	0.22	0.36	0.03	0.05
	(0.3)	(0.3)	(2.2)	(3.6)	(0.7)	(0.1)	(0.07)	(0.09)	(0.01)	(0.02)
3	2.9	2.8	34.6	36.2	1.2	1.3	0.34[a]	0.55[b]	0.07	0.05
	(0.2)	(0.2)	(5.3)	(5.0)	(0.1)	(0.1)	(0.08)	(0.07)	(0.02)	(0.01)
6	2.9	2.8	32.8	32.9	2.6	2.6	0.41	0.51	0.07	0.06
	(0.2)	(0.2)	(2.3)	(2.2)	(0.2)	(0.9)	(0.12)	(0.04)	(0.02)	(0.01)
9	2.8	2.9	33.7	32.3	3.7[b]	2.4[a]	0.26	0.34	0.06	0.11
	(0.3)	(0.4)	(4.0)	(3.6)	(0.2)	(0.6)	(0.08)	(0.12)	(0.02)	(0.04)
13	2.6	2.8	36.1	33.9	3.5	3.3	0.27	0.36	0.11	0.17
	(0.3)	(0.2)	(3.0)	(3.7)	(1.1)	(0.7)	(0.06)	(0.12)	(0.04)	(0.03)
16	2.7	2.6	37.1	33.9	1.3[a]	2.0[b]	0.30	0.36	0.20	0.27
	(0.2)	(0.3)	(2.2)	(2.8)	(0.1)	(0.4)	(0.03)	(0.08)	(0.04)	(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.

Table 3. Comparative endogenous antioxidant assessment (mg/kg fish muscle)[§] in farmed rainbow trout after slaughtering and chilled storage in FI and OFI.[$]

Icing time [days]	Astaxanthin		α-Tocopherol		γ-Tocopherol		δ-Tocopherol	
	FI	OFI	FI	OFI	FI	OFI	FI	OFI
1	1.9	2.1	12.9	12.6	2.3	2.2	0.31	0.37
	(0.3)	(0.2)	(2.5)	(3.3)	(0.3)	(0.7)	(0.06)	(0.07)
3	1.8	1.7	13.2	15.3	2.3	2.8	0.40	0.29
	(0.3)	(0.3)	(2.0)	(2.7)	(0.4)	(0.6)	(0.03)	(0.04)
6	1.6	2.4	12.4	13.5	2.2	2.4	0.39	0.29
	(0.3)	(0.4)	(1.8)	(1.9)	(0.2)	(0.4)	(0.03)	(0.06)
9	1.9	2.3	14.1	14.4	2.2	2.5	0.42	0.46
	(0.4)	(0.3)	(1.7)	(3.3)	(0.2)	(0.4)	(0.06)	(0.06)
13	2.4	1.7	9.8	16.6	2.8	2.7	0.45	0.32
	(0.4)	(0.4)	(2.3)	(3.0)	(0.4)	(0.8)	(0.09)	(0.05)
16	2.2	2.1	8.6	12.7	2.1	2.2	0.29	0.28
	(0.3)	(0.3)	(3.2)	(2.6)	(0.2)	(0.5)	(0.05)	(0.03)

[§] Average values of three ($n = 3$) independent determinations. Standard deviations are indicated in brackets.
[$] For each parameter, no significant differences ($p > 0.05$) between both icing conditions nor as a result of the frozen storage time were detected.

compared to their counterparts under FI, with 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 conditions when compared to its counterpart under FI treatment; however, this 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 occur during the 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 occur as a result of bacterial activity. The present results on FFA formation show values below 1% in all cases, which can be considered as lower than those obtained for most marine lean fish species when kept under chilling conditions [6, 34]. In addition, a higher FFA formation during chilled storage could be observed for the same farmed species [20]. Accord-

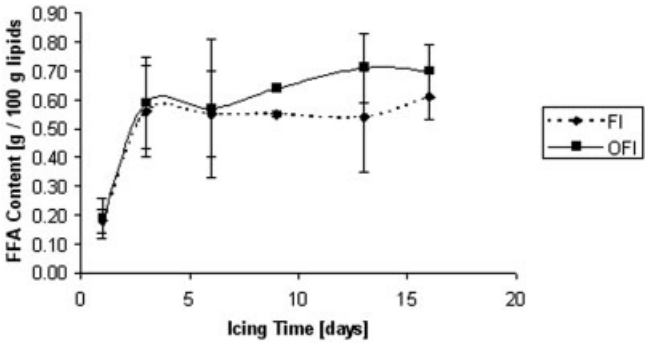

Figure 2. Comparative FFA formation in farmed rainbow trout muscle after slaughtering and chilled storage in FI and OFI. * Mean values of three ($n = 3$) independent determinations. Standard deviations are indicated by bars.

ingly, a low lipid hydrolytic activity is concluded to occur after slaughtering and chilled storage of the present freshwater 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 catalytic effect of the carboxyl group on the formation of free radicals by the decomposition of hydroperoxides [43]. In this sense, Han and Liston [44] provided marked support for a straight correlation between lipid peroxidation and PL hydrolysis in frozen (–10 °C) rainbow trout.

3.4 Lipid oxidation

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

Peroxide formation was very low in all cases (PV range: 1.0–4.0) (Table 2), similar to the results obtained by applying FI to a lean fish species [6] and lower than the peroxide development observed in a lean fish species chilled under flake icing [34]. In the present work, a clear tendency with time could not be observed for individuals treated under both icing conditions ($p > 0.05$); only some small differences between the icing conditions could be assessed for the PV, with these, however, not leading to any conclusions concerning the effect of ozone presence, according to previous research [40]. Low scores obtained in the present experiment for the peroxide formation agree with the retention of AX content, according to the scavenger role of this carotenoid compound in the very early stages of lipid oxidation [17].

More important than primary oxidation was secondary lipid oxidation development (Table 2). Thus, TBARS values ranged between 0.15 and 0.60, which can be considered similar to scores found for a medium-fat fish [8], but far lower than those reported for a fatty fish [7], all stored under FI conditions. A definite effect of icing time could not be observed ($p > 0.05$) for both icing systems in the present work. Comparison between both slaughtering and storage conditions showed higher mean values for individuals under OFI conditions, although significant differences could only be observed at days 1 and 3. A slight pro-oxidant effect of the presence of ozone could be concluded, which does not agree with previous work [40]. In that study, a lower TBARS formation was observed in turbot chilled under OFI conditions when compared to its counterpart under FI treatment; however, this result was obtained in a 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 any differences ($p > 0.05$) between both icing conditions (Table 2). However, higher mean values for individuals slaughtered and kept under OFI conditions were obtained in the 9–16-day period, according to the above-mentioned TBARS formation. An increasing ($p < 0.05$) FR tendency with icing time could be detected for both storage systems, which can be explained as a result of increasing lipid damage. However, the FR values obtained for both kinds of fish individuals can be considered as notably low, according to previous research on chilled 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, 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 between these and 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].

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

Table 4. Comparative sensory evaluation of farmed rainbow trout after slaughtering and chilled storage in FI and OFI.[§]

Icing time [days]	Attribute									
	Skin		Eyes		External odour		Gills		Consistency	
	FI	OFI	FI	OFI	FI	OFI	FI	OFI	FI	OFI
1	E	E	E	E	E	E	E	E	E	E
3	E	E	E	E	E	E	E	E	A	A
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.

valuable effect can be attributed to its strong antimicrobial power [9].

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

3.6 Colour analysis

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

Values for the lightness (L⋆) parameter did not provide significant differences ($p > 0.05$) as a result of the icing time, and ranged between 54.5 and 64.4. Individuals treated under OFI condition showed some higher values (days 9 and 13) than their counterparts treated with the FI system; however, this tendency was not maintained at the end of the experiment. Concerning the redness (a⋆) and yellowness (b⋆) parameters, differences could not be obtained ($p > 0.05$) as a result of icing time, nor by comparing both icing conditions. These results agree with the previously mentioned data showing no changes for the AX content (Table 3).

Previous research related to 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 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 the two parameters (TBARS formation and a⋆) provided a definite tendency with icing time.

Table 5. Comparative assessment of colour changes[§] in farmed rainbow trout after slaughtering and chilled storage in FI and OFI.[$]

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

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 relatively stable lipid composition, so that low lipid damage development and marked sen-

sory quality retention were obtained. Thus, low lipid hydrolysis and oxidation development could be observed, which was followed by the absence of lipid composition (PUFA, PL and endogenous antioxidants) and instrumental colour changes. Concerning the sensory acceptance, remarkably good quality and shelf life times were obtained. According to present demands on the quality of freshwater farmed fish species, it is concluded that FI as such, or including the presence of ozone, can be considered as ideal strategy to be employed as slaughtering and chilling system 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, the oxidation values reached by individuals kept under OFI conditions cannot be considered as particularly high.

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Conflict of interest statement

The authors have declared no conflict of interest.

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