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Application of Active Packaging in Refrigerated Rainbow Trout (*Oncorhynchus mykiss*) Fillets Treated with UV-C Radiation

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Abstract: This study investigated the effects of oxygen-scavenger packaging and UV-C radiation at two doses, alone or in combination, on lipid oxidation (malondialdehyde levels), protein oxidation (carbonyl content), instrumental color and texture parameters in rainbow trout fillets stored at 4 ± 1 °C for 9 days. The treatments were AP (air packaging), OSP (oxygen-scavenger packaging), AUV1 (air packaging + UV-C at 0.102 J/cm^2), OSUV1 (oxygen-scavenger packaging + UV-C at 0.102 J/cm^2), AUV3 (air packaging + UV-C at 0.301 J/cm^2), and OSUV3 (oxygen-scavenger packaging + UV-C at 0.301 J/cm^2). Lipid oxidation, protein oxidation, lightness and yellowness increased, while redness, hardness and chewiness decreased during storage in all treatments ($p < 0.05$). OSP, OSUV1 and OSUV3 exhibited lower yellowness, total color difference (ΔE), lipid and protein oxidation, and higher redness, hardness and chewiness than air packaging (AP; $p < 0.05$), being similar to each other concerning these parameters throughout storage ($p > 0.05$). AUV3 showed higher lipid oxidation, protein oxidation, yellowness, ΔE, and lower redness, hardness and chewiness followed by AUV1 than AP ($p < 0.05$). UV-C at these doses was not adequate for refrigerated trout fillets by inducing oxidative degradation. O_2 scavenger was effective in preventing the adverse effects from storage period and UV-C, independently of the dose, and could be a simple and powerful alternative to make feasible the industrial application of UV-C at 0.102 and 0.301 J/cm^2 in refrigerated rainbow trout fillets, which has proven antimicrobial effect and it is a promising non-thermal technology for the fish production chain.

Keywords: freshwater fish; oxygen scavenger; UV-C light; hurdle technology; oxidative degradation; storage quality

1. Introduction

Rainbow trout (*Oncorhynchus mykiss*) is one of the most important freshwater fish species for an increase in the world trade of fish and fish products [1]. Nevertheless, trout is highly susceptible to oxidative degradation due to high unsaturated and saturated fatty acids ratio (5.23), high amount of protein (20%) and low stability related to conformation and structure of the hemoglobin [2–4].

The oxidative degradation is the main non-microbiological reason to off-flavor, discoloration, loss of texture, and formation of toxic compounds in the postmortem period, which are determinant factors

to shorter shelf life, rapid loss of overall quality and consumer rejection [5,6]. Currently, the loss of quality from capture to final consumption results in a discard of 27% of the fish, and it is the main limiting factor to increase worldwide commercialization and consumption of fish [1]. To overcome this challenge, the United Nations Food and Agriculture Organization [1] suggests studies concerning viable preservation technologies aiming to achieve a maximum shelf life without compromise the original quality parameters of fish, which is one of the most challenges for the scientific community and food industry by the use of a single method. In this way, studies about combined preservation treatments have increased [7–10].

UV-C radiation is a promising non-thermal technology for the fish production chain. Beyond the proven efficacy to decrease the bacterial growth rate during refrigerated storage of fish species, UV-C has low cost, does not generate toxic residues and can be easily implemented in the fishery industry without changing the production flow [9,11,12]. The effects of UV-C depend mainly on the dose used, food composition, type and load of microorganisms into the food matrix [11–14]. Nevertheless, in general, UV-C doses (0.10–0.30 J/cm^2) needed to reach a shelf life extension of 2–6 days in refrigerated fish species, including rainbow trout, lead to one or more oxidative damages by producing reactive oxygen species (ROS) compromising overall fish quality, which is the main limiting factor for the use of UV-C at industrial scale [9,12,13,15,16].

O_2 scavenger or O_2 absorber has been used preferentially to vacuum packaging (VP) and modified-atmosphere packaging (MAP) due to its ability to reduce and maintain the O_2 concentration inside the package at levels less than 0.01% being effective against oxidative degradation from ROS and growth of obligate aerobic microorganisms during the entire storage period [10,17,18]. O_2 scavengers contain compounds in powder such as ascorbic acid, catechols, sulfites, and mainly iron and ferrous oxide [18], which result in a constant oxygen consumption by oxidation of ferrous oxide (FeO) in ferric oxide (Fe_2O_3) [17,18]. Furthermore, O_2 scavengers are mainly sold in sachet form at low cost and do not require the use of equipment. At the present moment, it is known that O_2 scavenger extends the shelf life in 5–7 days while prevents the oxidation during refrigerated storage of fish species, including rainbow trout [7,9,19]. Therefore, O_2 scavenger could be a simple and effective alternative to mitigate the adverse effects of UV-C radiation.

Despite these facts, there is only one recent study evaluating the combined effect of O_2 scavenger and UV-C radiation in tilapia fillets stored under refrigeration [9], however rainbow trout is more prone to oxidative degradation than tilapia [2,3,13]. In this context, this study aimed to investigate the effect of oxygen-scavenger packaging and two UV-C doses (0.102 and 0.301 J/cm^2), alone or in combination, on oxidative stability, instrumental color and texture parameters of rainbow trout (*Oncorhynchus mykiss*) fillets stored at 4 ± 1 °C for 9 days.

2. Materials and Methods

2.1. Experimental Design

One hundred and twenty skinless fresh fillets from 120 different farmed rainbow trout (*Oncorhynchus mykiss*) were purchased at a local fish farm in Rio de Janeiro, Brazil (22°41′57″ S 42°52′40″ W) and then were immediately transported in styrofoam boxes containing ice (0 °C) to the laboratory. Fillets (114.76 g ± 5.25 g each) were individually placed in nylon/polyethylene packages (80 µm thickness, 22 cm height and 15 cm width) with the following barrier properties according to manufacturer's specifications: O_2 transmission rate (OTR) of 66.31 cm^3/m^2/day and water-vapor transmission rate (WVTR) of 4.91 g/m^2/day at 23 °C and 50% relative humidity (Gabrilina, São Paulo, Brazil). Then, packages were added or not of one oxygen absorber sachet and then were sealed (Engevac, São Paulo, Brazil). Fillets were randomly distributed into six different treatments according to packaging systems (air or oxygen scavenger) and UV-C doses (0.102 or 0.301 J/cm^2), which are shown in Table 1. Immediately after each treatment, trout fillets were stored at 4 ± 1 °C and analyzed in duplicate for lipid oxidation and protein oxidation, and in quadruplicate for instrumental color

parameters and texture profile analysis on days 0, 3, 6 and 9. Each treatment was composed of 20 packages (5 replicates × 4 days of storage; $n = 5$) with a headspace of 45.54 ± 1.15 mL. The headspace was calculated by the difference of the total volume of the package and the sample volume of 114.76 g ± 5.25 g of trout fillet. The days of storage were chosen based on the shelf life of refrigerated trout fillets treated singly with either O_2 absorber [7] or UV-C dose at 0.110 J/cm^2 [12]. Air packaging was chosen instead of vacuum packaging to evaluate the effects of the two UV-C doses and O_2 scavenger in the worst scenario for oxidative processes.

Table 1. Treatments applied in rainbow trout (*Oncorhynchus mykiss*) fillets stored at 4 ± 1 °C for 9 days.

Treatments	Code	Description [¥]
Air packaging	AP	The fillet was placed in the package, which was immediately sealed
Oxygen-scavenger packaging	OSP	The fillet was placed in the package, one oxygen absorber was inserted, and then it was immediately sealed
Air packaging + UV-C	AUV1	Packed fillet (AP) was submitted to UV-C at 0.102 J/cm^2
Oxygen-scavenger packaging + UV-C	OSUV1	Packed fillet (OSP) was submitted to UV-C at 0.102 J/cm^2
Air packaging + UV-C	AUV3	Packed fillet (AP) was submitted to UV-C at 0.301 J/cm^2
Oxygen-scavenger packaging + UV-C	OSUV3	Packed fillet (OSP) was submitted to UV-C at 0.301 J/cm^2

[¥] All fillets were packed in nylon/polyethylene packages (Gabrilina, São Paulo, Brazil).

2.2. Oxygen Scavenger Packaging

Before sealing, one oxygen absorber sachet (Ageless SS-50; Mitsubishi Gas Chemical Co., Inc., Tokyo, Japan) was placed inside each package (OSP, OSUV1 and OSUV3). According to manufacturer's manual, this O_2 absorber sachet has the capacity of O_2 absorption of 50 mL, and its use reduces and maintains O_2 concentration at very low levels (<0.01%) inside the package due to spontaneous iron oxidation through the conversion of ferrous oxide (FeO) to ferric oxide (Fe_2O_3).

2.3. UV-C Treatment

UV-C apparatus used in this study was developed by Lázaro et al. [20]. This equipment is a stainless steel barrel-shaped chamber containing twelve UV-C lamps (six of 30 W and six of 55 W; OSRAM HNS, OFR, Munich, Germany). After package sealing, trout fillets (AUV1, OSUV1, AUV3 and OSUV3) were individually put on the center of the UV-C equipment at a distance of 14 cm from the lamps. In each UV-C exposure, a UV radiometer (MRUR-203, Instrutherm Ltda., São Paulo, Brazil) wrapped with the same sample packaging was placed next to the sample to be irradiated to measure the UV intensities every 5 s until reaching the dose of 0.102 ± 0.001 J/cm^2 (AUV1 and OSUV1) or 0.301 ± 0.001 J/cm^2 (AUV3 and OSUV3). The UV-C dose of 0.102 J/cm^2 was chosen due to its effectiveness in extending the shelf life with no effect on lipid oxidation in vacuum-packed rainbow trout fillets stored under refrigeration for 22 days, which was attributed to low UV-C dose and very low levels of O_2 [12]. On the other hand, the UV-C dose of 0.301 J/cm^2 was chosen because the effect of this dose in refrigerated trout fillets is still unknown.

2.4. Determination of Lipid Oxidation

Lipid oxidation was evaluated by quantifying malondialdehyde (MDA) levels through thiobarbituric acid-reactive substances (TBARS) method, as described by Yin et al. [21] with slight adaptations [22]. Briefly, fillets were added of 11% trichloroacetic acid aqueous solution (TCA; *w*/*v*), and it was homogenized using an Ultra Turrax 18 basic (IKA, Wilmington, NC, USA). Then, the content was centrifuged at 15,000× *g* for 15 min at 4 °C, the supernatant was added of 20 mM thiobarbituric acid (TBA) aqueous solution (*w*/*v*), the mixture was vortexed, and incubated for 20 h in dark condition. The absorbance values were measured at 532 nm on a UV-1800 spectrophotometer

(Shimadzu, Kyoto, Japan), and their conversion in mg MDA/kg fish tissue was obtained from a calibration curve (R^2 = 0.999) built with seven MDA concentrations ranging from 1 to 500 µmol.

2.5. Determination of Protein Oxidation

Protein oxidation was quantified by the reaction between carbonyl groups and 2, 4 dinitrophenylhydrazine (DNPH) forming protein hydrazones according to methodology of Oliver et al. [23] modified by Mercier et al. [24] and Armenteros et al. [25] using a UV-1800 spectrophotometer (Shimadzu, Kyoto, Japan). Briefly, the fillet was added of 0.15 M potassium chloride solution (pH 7.4), it was homogenized using an Ultra Turrax 18 basic (IKA, Wilmington, NC, USA), and the homogenate was precipitate from the addition of 10% trichloroacetic acid (TCA; *w*/*v*) and centrifugation (5000× *g* for 5 min at 4 °C). Then, the precipitate was added of DNPH and, after precipitation using 10% TCA (*w*/*v*) and centrifugation (11,000× *g* for 10 min at 4 °C), it was washed three times with ethanol/ethyl acetate solution (1:1; *v*/*v*) and solubilized with 6 M guanidine hydrochloride in 20 mM sodium phosphate buffer (pH 6.5). For protein quantification, the absorbance values were read at 280 nm and converted in mg by a calibration curve (R^2 = 0.999) built with five different concentrations of BSA (bovine serum albumin) ranging from 0.1 to 1.0 mg. For carbonyl quantification, the absorbance values were measured at 370 nm, and the carbonyl content was expressed as nmol carbonyls/mg protein using an absorption coefficient of 21.0/mM/cm for protein hydrazones.

2.6. Instrumental Color Measurements

Immediately after removing the samples from the packages, lightness (L^*), redness (a^*) and yellowness (b^*) values were measured at four random locations on the surface of each trout fillet using a Minolta CM-600D portable colorimeter (Minolta Camera Co., Osaka, Japan) set at an illuminant A, 8 mm-diameter aperture, and 10° standard observer [26]. Furthermore, the total color difference (ΔE) between the last and first day of refrigerated storage was calculated for all treatments according to the following equation [26]:

$$\Delta E_{9\text{-}0} = [(L^* - L_0{}^*)^2 + (a^* - a_0{}^*)^2 + (b^* - b_0{}^*)^2]^{1/2} \quad (1)$$

2.7. Instrumental Texture Profile

A TA.XT plus Texture Analyser (Stable Micro Systems, Surrey, UK) coupled a cylindrical P/36R probe (36 mm) was used to determine the texture profile analysis (TPA) in four equal pieces ($2 \times 2 \times 2$ cm^3) from each trout fillet. TPA was performed using two compression cycles of 50% with an interval of 5 s between them and pre-test, test, and post-test speeds of 1 mm/s [27]. Hardness, chewiness, cohesiveness, springiness, and resilience parameters were obtained from a force-time curve created by Texture Exponent software (Stable Micro Systems, Surrey, UK).

2.8. Statistical Analyses

The experiment followed a fully randomized design (n = 5). Two-way ANOVA was used to identify differences between treatments (AP, OSP, AUV1, OSUV1, AUV3 and OSUV3) and days of storage (0, 3, 6 and 9) for all evaluated parameters. When F value was significant, the differences were determined by Tukey's test. Pearson's correlation was used to identify correlations among all parameters. All statistical analyses were carried out at 95% confidence level using XLSTAT software (Addinsoft, New York, NY, USA).

3. Results and Discussion

3.1. Lipid Oxidation

MDA levels increased over the storage period in all treatments, however it was more pronounced in AUV1 and AUV3, and less pronounced in OSP, OSUV1 and OSUV3 ($p < 0.05$; Table 2). On day 0,

no difference was observed in MDA levels among all treatments. On days 3, 6 and 9, AUV3 exhibited the highest MDA levels followed by AUV1 in comparison to AP ($p < 0.05$), indicating that UV-C radiation affected the lipid oxidation in a dose-dependent manner. On the other hand, OSP, OSUV1 and OSUV3 had similar MDA levels ($p > 0.05$), which were lower than AP on days 3, 6 and 9 ($p < 0.05$; Table 2).

Table 2. Oxidative stability of rainbow trout (*Oncorhynchus mykiss*) fillets non- and treated with oxygen scavenger packaging (OSP) and ultraviolet radiation (UV-C) stored at 4 ± 1 °C for 9 days.

Parameters	Treatments [€]	Days of Storage			
		0	3	6	9
Lipid oxidation (mg MDA [¥]/kg fish)	AP	0.117 ± 0.010 ^{az}	0.429 ± 0.038 ^{cy}	0.662 ± 0.045 ^{cx}	0.991 ± 0.069 ^{cw}
	OSP	0.121 ± 0.010 ^{az}	0.224 ± 0.017 ^{dy}	0.479 ± 0.035 ^{dx}	0.757 ± 0.061 ^{dw}
	AUV1	0.120 ± 0.011 ^{az}	0.542 ± 0.051 ^{by}	0.758 ± 0.059 ^{bx}	1.157 ± 0.072 ^{bw}
	OSUV1	0.122 ± 0.012 ^{az}	0.227 ± 0.015 ^{dy}	0.476 ± 0.037 ^{dx}	0.759 ± 0.069 ^{dw}
	AUV3	0.117 ± 0.011 ^{az}	0.671 ± 0.059 ^{ay}	0.873 ± 0.070 ^{ax}	1.343 ± 0.079 ^{aw}
	OSUV3	0.120 ± 0.011 ^{az}	0.231 ± 0.021 ^{dy}	0.484 ± 0.044 ^{dx}	0.774 ± 0.047 ^{dw}
Protein oxidation (nmol carbonyl/ mg protein)	AP	1.33 ± 0.17 ^{az}	2.48 ± 0.23 ^{cy}	3.46 ± 0.31 ^{cx}	4.42 ± 0.30 ^{cw}
	OSP	1.30 ± 0.15 ^{az}	1.88 ± 0.19 ^{dy}	2.67 ± 0.21 ^{dx}	3.58 ± 0.36 ^{dw}
	AUV1	1.33 ± 0.15 ^{az}	2.70 ± 0.26 ^{by}	4.08 ± 0.39 ^{bx}	4.79 ± 0.43 ^{bw}
	OSUV1	1.33 ± 0.08 ^{az}	1.84 ± 0.19 ^{dy}	2.60 ± 0.26 ^{dx}	2.53 ± 0.34 ^{dw}
	AUV3	1.35 ± 0.14 ^{az}	3.34 ± 0.24 ^{ay}	4.62 ± 0.28 ^{ax}	5.16 ± 0.24 ^{aw}
	OSUV3	1.36 ± 0.12 ^{az}	1.90 ± 0.14 ^{dy}	2.66 ± 0.24 ^{dx}	3.54 ± 0.33 ^{dw}

[€] AP (air packaging); OSP (oxygen scavenger packaging); AUV1 (air packaging + UV-C at 0.102 J/cm^2); OSUV1 (oxygen scavenger packaging + UV-C at 0.102 J/cm^2); AUV3 (air packaging + UV-C at 0.301 J/cm^2); and OSUV3 (oxygen scavenger packaging + UV-C at 0.301 J/cm^2). [¥] MDA—malonaldehyde. Results are expressed as means ± standard deviation ($n = 5$). ^{a,b,c,d} Means with different superscripts indicate significant differences ($p < 0.05$) amongst treatments. ^{w,x,y,z} Means with different superscripts indicate significant differences ($p < 0.05$) amongst days of storage.

Lipid oxidation is initiated by factors such as temperature, light, enzymes, reactive oxygen species (ROS) or presence of pro-oxidants, which remove a hydrogen atom from an unsaturated fatty acid and, in the presence of O_2, it results in a complex free-radical chain reaction [6,28]. It leads to formation of primary compounds like peroxide radicals (ROO·) and hydroperoxides (ROOH), which due to their instability result in the formation of secondary compounds such as aldehydes, ketones and hydrocarbons, which are stable and responsible for off-flavor and meat discoloration during refrigerated storage, mainly the aldehydes [6,28,29]. Rainbow trout is a farmed freshwater fish species with high susceptibility to lipid oxidation due to its fatty acid profile, which contains approximately 82.60% of unsaturated fatty acids and 15.80% of saturated fatty acids [2]. These facts may explain the increase of lipid oxidation over the storage period found in this study. Similarly, Rodrigues et al. [12] and Sáez et al. [30] observed an increase in the MDA levels in farmed rainbow trout fillets stored at 4 °C under aerobic conditions for 22 and 15 days, respectively.

The increase of MDA levels by both UV-C doses may be attributed to the pro-oxidant effect of UV-C radiation generating ROS and accelerating the lipid oxidation [11]. In agreement with our findings, Monteiro et al. [9] observed a dose-dependent effect of two UV-C doses (0.10 and 0.30 J/cm^2) on an increase of MDA levels in refrigerated tilapia fillets for 23 days. Likewise, Molina et al. [16] reported the same effect on lipid oxidation due to the application of UV-C doses at 0.80 and 1.60 J/cm^2 in sea bass fillets stored at 4 °C for 11 days. The effectiveness of the O_2 scavenger against lipid oxidation is due to its ability to reduce the O_2 concentration inside the package (<0.01%) and to minimize the free-radical chain reaction induced by ROS [17,18,31]. Similarly, O_2 scavenger was able to prevent the lipid oxidation of fish species stored under refrigeration, such as rainbow trout fillets [7], fresh cobia steaks [10], Indian oil sardine [19], and dried sardine [32].

Regarding combined treatments, there is a lack of studies concerning the effect of O_2 scavenger in foods in the worst scenario, like in fish species subjected to oxidation-inducing treatments. Monteiro et al. [9] observed that O_2 scavenger prevented the lipid oxidation induced by UV-C radiation

in tilapia fillets stored under refrigeration, regardless of the dose (0.10 and 0.30 J/cm^2), corroborating with our results. Nevertheless, it is worth highlighting that, when compared with tilapia, trout flesh has a higher amount of unsaturated fatty acids and is more biochemically unstable, and therefore is more prone to lipid oxidation [2,3,13].

3.2. Protein Oxidation

Carbonyl content showed the same behavior observed for MDA levels considering the storage period and the differences among all treatments ($p < 0.05$; Table 2).

Protein oxidation follows a similar pathway to lipid oxidation generating a free-radical chain reaction through abstraction of a hydrogen atom from a protein, mainly from functional groups into the side chains of amino acids [33,34]. Depending on the oxidized amino acid, there is the formation of carbonyl residues (e.g., oxidation of lysine, arginine and proline), cross-linking and sulfur-containing residues (e.g., oxidation of cysteine and methionine), which result in adverse effects on color and texture of meat products [5,33]. In this way, the increase of carbonyl content occurs naturally during refrigerated storage of fish species, which is well known in trout fillets [35,36] and agrees with our findings.

The differences between treatments may be explained by the same reasons as those mentioned for lipid oxidation in association with the fact that protein and lipid oxidation are concomitant reactions [8,28]. MDA from lipid oxidation can bind to amino acid residues into myoglobin and to some sites into myofibrillar proteins exposing it to the oxidizing environment and making it more susceptible to protein oxidation [29,37]. On the other hand, protein oxidation releases large amounts of free iron, which are pro-oxidant agents for lipid oxidation [28,37]. In the present study, a positive correlation was observed between protein and lipid oxidation ($r = 0.986$, $p = 0.000$), which reinforces our findings.

Although protein oxidation is recognized as a good indicator of fish quality, the isolated effect of UV-C radiation and, mainly of the O_2 scavenger on carbonyl content of fish species is scarce in the literature. Similar to our findings, UV-C doses at 0.10 and 0.30 J/cm^2 increased carbonyl levels in a dose-dependent manner in tilapia fillets stored at 4 °C [9,13]. Likewise, O_2 scavenger decreased the formation of carbonyl groups in tilapia fillets stored under aerobic refrigerated conditions for 23 days [9]. Furthermore, protein oxidation induced by two UV-C doses (0.10 and 0.30 J/cm^2) was equally suppressed by O_2 scavenger during refrigerated storage of tilapia fillets [9], which has lower oxidative potential than the target fish species of this study [2,3,13].

3.3. Instrumental Color Parameters

L^* values increased over the storage period in all treatments ($p < 0.05$), and no difference was observed between them on days 0, 3, 6 and 9 ($p > 0.05$; Table 3). The increase of L^* values during storage has been related to protein denaturation, which leads to exposure of hydrophobic groups and subsequent water loss resulting in changes in meat surface reflectance [38]. The increase of L^* values as the increase of storage period was found in other refrigerated freshwater fish species [9,13,39], including rainbow trout [30]. Likewise, similar UV-C doses (0.10 and 0.30 J/cm^2) and O_2 scavenger, alone or in combination, did not affect L^* values of tilapia fillets stored under refrigeration [8,9,13], corroborating with our results.

Table 3. *L** (lightness), *a** (redness) and *b** (yellowness) of rainbow trout (*Oncorhynchus mykiss*) fillets non- and treated with oxygen scavenger packaging (OSP) and ultraviolet radiation (UV-C) stored at 4 ± 1 °C for 9 days.

Parameters	Treatments [€]	Days of Storage			
		0	3	6	9
*L**	AP	53.51 ± 0.98 ^{ay}	57.65 ± 1.86 ^{ax}	59.48 ± 2.20 ^{ax}	65.45 ± 2.67 ^{aw}
	OSP	54.00 ± 1.95 ^{ay}	57.83 ± 1.86 ^{ax}	59.21 ± 2.36 ^{ax}	64.77 ± 2.38 ^{aw}
	AUV1	53.83 ± 2.12 ^{ay}	58.05 ± 1.52 ^{ax}	59.47 ± 2.39 ^{ax}	65.01 ± 2.79 ^{aw}
	OSUV1	53.61 ± 1.90 ^{ay}	57.09 ± 2.04 ^{ax}	59.09 ± 1.81 ^{ax}	64.38 ± 2.14 ^{aw}
	AUV3	53.45 ± 2.59 ^{ay}	58.11 ± 2.02 ^{ax}	60.07 ± 1.31 ^{ax}	65.35 ± 2.59 ^{aw}
	OSUV3	53.37 ± 1.89 ^{ay}	57.20 ± 1.90 ^{ax}	59.27 ± 1.90 ^{ax}	64.52 ± 2.63 ^{aw}
*a**	AP	5.09 ± 0.25 ^{aw}	4.05 ± 0.29 ^{bx}	3.34 ± 0.30 ^{by}	2.66 ± 0.19 ^{bz}
	OSP	5.21 ± 0.13 ^{aw}	4.76 ± 0.15 ^{ax}	4.29 ± 0.22 ^{ay}	3.70 ± 0.15 ^{az}
	AUV1	5.11 ± 0.25 ^{aw}	3.62 ± 0.17 ^{cx}	2.67 ± 0.24 ^{cy}	2.16 ± 0.13 ^{cz}
	OSUV1	5.26 ± 0.25 ^{aw}	4.80 ± 0.13 ^{ax}	4.32 ± 0.26 ^{ay}	3.65 ± 0.18 ^{az}
	AUV3	5.10 ± 0.28 ^{aw}	3.02 ± 0.21 ^{dx}	2.07 ± 0.15 ^{dy}	1.67 ± 0.09 ^{dz}
	OSUV3	5.29 ± 0.29 ^{aw}	4.79 ± 0.17 ^{ax}	4.23 ± 0.27 ^{ay}	3.68 ± 0.21 ^{az}
*b**	AP	7.02 ± 0.57 ^{az}	8.84 ± 0.45 ^{cy}	9.74 ± 0.48 ^{cx}	10.96 ± 0.57 ^{cw}
	OSP	7.04 ± 0.58 ^{ay}	8.15 ± 0.41 ^{dx}	8.82 ± 0.34 ^{dw}	9.34 ± 0.33 ^{dw}
	AUV1	7.08 ± 0.61 ^{az}	9.87 ± 0.31 ^{by}	10.46 ± 0.42 ^{bx}	11.72 ± 0.44 ^{bw}
	OSUV1	7.06 ± 0.50 ^{ay}	8.29 ± 0.44 ^{dx}	8.88 ± 0.19 ^{dw}	9.38 ± 0.31 ^{dw}
	AUV3	7.18 ± 0.64 ^{az}	10.76 ± 0.43 ^{ay}	11.50 ± 0.43 ^{ax}	12.33 ± 0.53 ^{aw}
	OSUV3	7.11 ± 0.50 ^{ay}	8.26 ± 0.46 ^{dx}	8.76 ± 0.48 ^{dx}	9.40 ± 0.39 ^{dw}
		Total color difference [¥]			
$\Delta E_{9\text{-}0}$	AP	11.88 ± 0.79 ^{c}			
	OSP	10.70 ± 0.74 ^{d}			
	AUV1	13.71 ± 0.97 ^{b}			
	OSUV1	10.47 ± 0.28 ^{d}			
	AUV3	16.25 ± 1.18 ^{a}			
	OSUV3	10.64 ± 0.68 ^{d}			

[€] AP (air packaging); OSP (oxygen scavenger packaging); AUV1 (air packaging + UV-C at 0.102 J/cm^2); OSUV1 (oxygen scavenger packaging + UV-C at 0.102 J/cm^2); AUV3 (air packaging + UV-C at 0.301 J/cm^2); and OSUV3 (oxygen scavenger packaging + UV-C at 0.301 J/cm^2). [¥] $\Delta E_{9\text{-}0}$ indicates total color difference of a same sample between days 9 and 0. Results are expressed as means ± standard deviation (n = 5). ^{a,b,c,d} Means with different superscripts indicate significant differences ($p < 0.05$) amongst treatments. ^{w,x,y,z} Means with different superscripts indicate significant differences ($p < 0.05$) amongst days of storage.

Concerning redness (*a**) and yellowness (*b**), all treatments showed a decrease in *a** values and increase in *b** values throughout refrigerated storage, however the increase of *b** values was less pronounced in OSP, OSUV1 and OSUV3 ($p < 0.05$; Table 3). On day 0, all treatments had similar *a** and *b** values ($p > 0.05$). From day 3 to day 9, AUV3 exhibited lower *a** values and higher *b** values followed by AUV1 compared to AP ($p < 0.05$; Table 3), indicating a dose-dependent effect of UV-C on *a** and *b** coordinates as was found for MDA levels and carbonyl content. On the other hand, OSP, OSUV1 and OSUV3 showed higher *a** values and lower *b** values than AP ($p < 0.05$), and no difference was observed between them for both parameters on days 3, 6 and 9 ($p > 0.05$; Table 3). It suggests that O_2 scavenger prevented changes on *a** and *b** values, and suppressed it independently of the UV-C dose corroborating with results observed for MDA levels and carbonyl content.

The decrease of *a** values is associated to meat discoloration due to myoglobin (Mb) autooxidation that occurs when oxymyoglobin (OxyMb; bright red color) is transformed in metmyoglobin (MetMb; brown color) by conversion of the ferrous iron (Fe^{2+}) to ferric iron (Fe^{3+}) [37,40]. Instead, the increase of *b** values is related to the increase of the lipid oxidation in fish species over the storage period [9,41]. Furthermore, MDA may stimulate the formation and accumulation of MetMb due to its ability to

inactivate the metmyoglobin-reducing system and to form covalent interactions with Mb, which lead to changes in their primary structure and increase the susceptibility of the myoglobin to oxidation [29,37].

In our study, decrease in a^* values and increase in b^* values corroborated with the increase in protein and lipid oxidation, which may be supported by our data from Pearson correlation. A negative correlation was observed between a^* values and MDA levels ($r = -0.996$; $p < 0.0001$) and a^* values and carbonyl content ($r = -0.993$; $p < 0.0001$), while a positive correlation was found for b^* values and MDA levels ($r = 0.983$; $p = 0.000$) and b^* values and carbonyl content ($r = 0.998$; $p < 0.0001$), explaining our findings for a^* and b^* values.

In agreement with our results, previous studies reported a decrease in a^* values during refrigerated storage of rainbow trout fillets [30] and pirarucu fillets [39], and also an increase of b^* values in pirarucu fillets [39] and tilapia fillets [8,9] under refrigerated storage. Also, similar to our results, Pedrós-Garrido et al. [42] and Lee et al. [43] observed that UV-C radiation at 0.19–0.38 J/cm^2 and 4.8 J/cm^2 enhanced meat discoloration by the decrease in a^* values and increase in b^* values in salmon fillets and semi-dried Pacific herring, respectively. Likewise, agreeing with our findings, previous studies have been confirmed the effectiveness of the O_2 scavenger in minimizing meat discoloration during the storage period of tilapia fillets [9] and dried sardine [32]. Among them, Monteiro et al. [9] observed that O_2 scavenger was able to equally inhibit meat discoloration induced by two UV-C doses (0.10 and 0.30 J/cm^2) in tilapia fillets stored at 4 °C for 23 days.

Regarding total color difference (ΔE), it indicates the magnitude of the color changes between treatments throughout refrigerated storage. In our study, ΔE was negatively correlated with a^* ($r = -0.965$; $p = 0.002$) and positively correlated with b^* values ($r = 0.936$; $p = 0.006$), MDA levels ($r = 0.984$; $p = 0.000$) and carbonyl content ($r = 0.945$; $p = 0.004$), which explain the differences found among treatments for ΔE over the storage period. Both UV-C at 0.102 J/cm^2 (AUV1) and 0.301 J/cm^2 (AUV3) exhibited higher color changes than AP in a dose-dependent manner ($p < 0.05$; Table 3). Moreover, O_2 scavenger, alone (OSP) and in combination with two UV-C doses (OSUV1 and OSUV3) showed lower color changes compared to AP ($p < 0.05$), and no difference was observed among O_2 scavenger treatments (OSP, OSUV1 and OSUV3) ($p > 0.05$; Table 3), reinforcing our findings for a^* and b^* coordinates, MDA levels, and carbonyl content.

According to Cruz-Romero et al. [44], ΔE values ranging from 6 to 12 correspond to great difference, while ΔE values equal or above 12 correspond to very great difference. Furthermore, ΔE values above 5 and 12 are equivalent to color changes visually perceptible to human eyes and untrained panelists, respectively [45]. Based on these classifications, color changes of the trout fillets submitted to 0.102 J/cm^2 (AUV1) and 0.301 J/cm^2 (AUV3) could be perceived and rejected by consumers.

3.4. Instrumental Texture Parameters

Hardness and chewiness decreased over the storage period in all treatments ($p < 0.05$; Table 4). On the first day of storage, all treatments had similar hardness and chewiness ($p > 0.05$). On days 3, 6, and 9, OSP, OSUV1 and OSUV3 demonstrated higher hardness and chewiness than other treatments ($p < 0.05$), and no difference was observed among O_2 scavenger treatments ($p > 0.05$). Overall, AUV3 showed lower hardness and chewiness, followed by AUV1 than AP from day 3 to day 9 ($p < 0.05$; Table 4). Cohesiveness, springiness and resilience was affected neither by storage period nor by treatments ($p > 0.05$; Table 4), which was already previously reported in other fish non- and treated with O_2 scavenger and/or UV-C radiation [9,30].

Table 4. Instrumental texture parameters of rainbow trout (*Oncorhynchus mykiss*) fillets non- and treated with oxygen scavenger packaging (OSP) and ultraviolet radiation (UV-C) stored at 4 ± 1 °C for 9 days.

Parameters	Treatments [€]	Days of Storage			
		0	3	6	9
Hardness (*N*)	AP	21.08 ± 1.85 ^{aw}	15.55 ± 1.44 ^{bx}	12.16 ± 0.89 ^{by}	10.32 ± 0.82 ^{bz}
	OSP	20.98 ± 2.00 ^{aw}	18.23 ± 1.47 ^{ax}	15.51 ± 1.35 ^{ay}	12.79 ± 1.20 ^{az}
	AUV1	21.13 ± 2.07 ^{aw}	12.88 ± 0.94 ^{cx}	10.08 ± 0.71 ^{cy}	7.86 ± 0.58 ^{cz}
	OSUV1	20.64 ± 1.61 ^{aw}	18.57 ± 1.30 ^{ax}	14.73 ± 1.28 ^{ay}	12.70 ± 1.11 ^{az}
	AUV3	21.14 ± 2.06 ^{aw}	10.31 ± 1.02 ^{dx}	8.09 ± 0.71 ^{dy}	7.06 ± 0.49 ^{cy}
	OPUV3	20.79 ± 1.70 ^{aw}	18.52 ± 1.10 ^{ax}	15.04 ± 1.12 ^{ay}	12.77 ± 1.15 ^{az}
Chewiness (*N* × mm)	AP	5.80 ± 0.45 ^{aw}	4.03 ± 0.35 ^{bx}	3.45 ± 0.33 ^{by}	2.85 ± 0.22 ^{bz}
	OSP	5.78 ± 0.52 ^{aw}	4.76 ± 0.32 ^{ax}	4.00 ± 0.32 ^{ay}	3.44 ± 0.31 ^{az}
	AUV1	5.73 ± 0.40 ^{aw}	3.43 ± 0.29 ^{cx}	2.75 ± 0.23 ^{cy}	2.07 ± 0.15 ^{cz}
	OSUV1	5.81 ± 0.35 ^{aw}	4.79 ± 0.41 ^{ax}	3.96 ± 0.34 ^{ay}	3.43 ± 0.29 ^{az}
	AUV3	5.83 ± 0.52 ^{aw}	2.81 ± 0.19 ^{dx}	2.17 ± 0.16 ^{dy}	1.76 ± 0.16 ^{cz}
	OPUV3	5.84 ± 0.44 ^{aw}	4.73 ± 0.46 ^{ax}	3.97 ± 0.26 ^{ay}	3.40 ± 0.31 ^{az}
Cohesiveness (ratio)	AP	0.330 ± 0.028 ^{aw}	0.339 ± 0.030 ^{aw}	0.337 ± 0.031 ^{aw}	0.342 ± 0.033 ^{aw}
	OSP	0.336 ± 0.028 ^{aw}	0.338 ± 0.029 ^{aw}	0.339 ± 0.029 ^{aw}	0.345 ± 0.027 ^{aw}
	AUV1	0.343 ± 0.029 ^{aw}	0.345 ± 0.033 ^{aw}	0.344 ± 0.033 ^{aw}	0.338 ± 0.028 ^{aw}
	OSUV1	0.343 ± 0.027 ^{aw}	0.345 ± 0.029 ^{aw}	0.342 ± 0.029 ^{aw}	0.337 ± 0.029 ^{aw}
	AUV3	0.337 ± 0.033 ^{aw}	0.340 ± 0.024 ^{aw}	0.339 ± 0.031 ^{aw}	0.342 ± 0.029 ^{aw}
	OPUV3	0.330 ± 0.031 ^{aw}	0.337 ± 0.030 ^{aw}	0.338 ± 0.028 ^{aw}	0.340 ± 0.030 ^{aw}
Springiness (ratio)	AP	0.532 ± 0.046 ^{aw}	0.542 ± 0.045 ^{aw}	0.549 ± 0.045 ^{aw}	0.541 ± 0.049 ^{aw}
	OSP	0.541 ± 0.049 ^{aw}	0.543 ± 0.045 ^{aw}	0.544 ± 0.040 ^{aw}	0.542 ± 0.040 ^{aw}
	AUV1	0.531 ± 0.049 ^{aw}	0.546 ± 0.050 ^{aw}	0.543 ± 0.044 ^{aw}	0.546 ± 0.042 ^{aw}
	OSUV1	0.539 ± 0.035 ^{aw}	0.544 ± 0.036 ^{aw}	0.549 ± 0.053 ^{aw}	0.547 ± 0.045 ^{aw}
	AUV3	0.539 ± 0.048 ^{aw}	0.544 ± 0.042 ^{aw}	0.550 ± 0.045 ^{aw}	0.545 ± 0.044 ^{aw}
	OPUV3	0.546 ± 0.042 ^{aw}	0.548 ± 0.033 ^{aw}	0.542 ± 0.045 ^{aw}	0.544 ± 0.046 ^{aw}
Resilience (ratio)	AP	0.124 ± 0.012 ^{aw}	0.119 ± 0.012 ^{aw}	0.123 ± 0.012 ^{aw}	0.118± 0.010 ^{aw}
	OSP	0.121 ± 0.011 ^{aw}	0.125 ± 0.012 ^{aw}	0.120 ± 0.011 ^{aw}	0.119 ± 0.010 ^{aw}
	AUV1	0.121 ±0.012 ^{aw}	0.124 ± 0.012 ^{aw}	0.120 ± 0.010 ^{aw}	0.121 ± 0.012 ^{aw}
	OSUV1	0.122 ± 0.012 ^{aw}	0.125 ± 0.010 ^{aw}	0.120 ± 0.010 ^{aw}	0.123 ± 0.010 ^{aw}
	AUV3	0.123 ± 0.010 ^{aw}	0.121 ± 0.012 ^{aw}	0.123 ± 0.012 ^{aw}	0.120 ± 0.009 ^{aw}
	OPUV3	0.121 ± 0.011 ^{aw}	0.123 ± 0.010 ^{aw}	0.122 ± 0.011 ^{aw}	0.121 ± 0.012 ^{aw}

[€] AP (air packaging); OSP (oxygen scavenger packaging); AUV1 (air packaging + UV-C at 0.102 J/cm^2); OSUV1 (oxygen scavenger packaging + UV-C at 0.102 J/cm^2); AUV3 (air packaging + UV-C at 0.301 J/cm^2); and OSUV3 (oxygen scavenger packaging + UV-C at 0.301 J/cm^2). Results are expressed as means ± standard deviation (n = 5). ^{a,b,c,d} Means with different superscripts indicate significant differences ($p < 0.05$) amongst treatments. ^{w,x,y,z} Means with different superscripts indicate significant differences ($p < 0.05$) amongst days of storage.

The decrease in hardness and chewiness during the storage of fish is associated with protein degradation due mainly to the action of endogenous and microbial proteases [46]. Similar results of hardness and chewiness were observed during refrigerated storage of rainbow trout fillets for 9 days [30] and tilapia fillets for 15 days [8]. Moreover, oxidative reactions may induce texture changes; however, it is a very complex issue, which is not yet fully understood. According to Li et al. [47], oxidized lipids and proteins generates free radicals leading mainly to changes in myofibrillar protein structure and exposure of its hydrophobic amino acids, which result in increased susceptibility to the action of proteolytic enzymes. In the present study, a positive correlation was found for hardness and chewiness (r = 0.991, p = 0.000), and negative correlations was found between hardness and MDA levels (r = −0.988; p = 0.000), hardness and carbonyl content (r = −0.991; p = 0.000), chewiness and MDA levels (r = −0.996; $p < 0.0001$), and chewiness and carbonyl content (r = −0.978; p = 0.001), which explain our results.

Although UV-C may reduce the microbial proteolysis due to antimicrobial effect, it may increase the endogenous proteolysis rate by inducing protein denaturation, which favors substrate-enzyme binding,

and by accelerating protein and lipid oxidation through ROS generation [8,11,12,48]. On the other hand, O_2 scavenger can minimize endogenous and microbial proteolysis. The O_2 scavenger act mainly against the growth of obligate aerobic microorganisms, which are represented by proteolytic spoilage bacteria in freshwater fish species stored under aerobic refrigerated conditions [10]. Furthermore, O_2 scavenger can reduce oxidative reactions induced by ROS by reducing O_2 levels inside the package [9,31]. Studies about the isolated or combined effect of UV-C and O_2 scavenger on texture parameters of meat under refrigerated storage are very scarce in the literature. Similar to our results, tilapia fillets treated with UV-C at 0.10 J/cm^2 showed lower hardness and chewiness than non-treated fillets during refrigerated storage [8]. Moreover, Molina et al. [16] observed that UV-C doses at 0.80 and 1.60 J/cm^2 accelerated collagen degradation in sea bass fillets stored under refrigeration. Regarding O_2 scavenger, Monteiro et al. [9] reported that it was able to delay the decrease of hardness and chewiness during refrigerated storage of tilapia fillets for 23 days. In this same study, O_2 scavenger was equally effective in retarding the decrease of hardness and chewiness in fillets subjected to two UV-C doses (0.10 and 0.30 J/cm^2).

4. Conclusions

Both UV-C doses (0.102 and 0.301 J/cm^2) accelerated lipid oxidation, protein oxidation, discoloration and softening of rainbow trout fillets in a dose-dependent manner. Thus their use is not recommended for refrigerated trout fillets. O_2 scavenger alone was equally effective as combined with each UV-C dose in preserving lipid oxidation, protein oxidation, discoloration and texture changes. Therefore, O_2 scavenger can be a simple and effective alternative to suppress the adverse effects of the UV-C radiation and make it viable for industrial applications to reach a longer shelf life while maintaining the original quality attributes characteristics in refrigerated rainbow trout fillets.

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