Combined effect of oxygen-scavenger packaging and UV-C radiation on shelf life of refrigerated tilapia (Oreochromis niloticus) fillets

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This study investigated the physicochemical, instrumental and bacterial parameters of tilapia fillets subjected to oxygen-scavenger packaging, alone or in combination with UV-C radiation at two doses (0.102 and 0.301 J/cm²), stored at 4 ± 1 °C for 23 days. The oxygen scavenger, both UV-C doses, and the oxygen scavenger combined with UV-C, independently of the dose, extended the shelf life in 5, 6 and 7 days, respectively, by decreasing the bacterial growth rate and the formation of degradation compounds (e.g., TVB-N and ammonia). Oxygen-scavenger packaging, alone or in combination with UV-C at 0.102 J/cm² and 0.301 J/cm² showed lower amounts of free amino acids (FAA; 34.39, 34.49 and 34.50 mg L-lysine/kg fish tissue, 3.63, 3.57 and 3.61 mg L- ornithine/kg fish tissue, 27.52, 27.63 and 27.67 mg L-arginine/kg fish tissue), biogenic amines (BA; 3.81, 3.87 and 3.89 mg cadaverine/kg fish tissue, 12.88, 12.91 and 12.86 mg putrescine/kg fish tissue, 2.41, 2.44 and 2.47 mg spermidine/kg fish tissue), redness (2.53, 2.55 and 2.59), yellowness (6.65, 6.69 and 6.72), lipid oxidation (1.52, 1.53 and 1.58 mg malondialdehyde/kg fish tissue) and protein oxidation (5.06, 5.11 and 5.18 nmol carbonyls/mg protein), with higher hardness (3273.41, 2652.98 and 2687.57 g) than control (air packaging; 41.97 mg L-lysine/kg fish tissue, 4.83 mg L- ornithine/kg fish tissue, 37.33 mg L-arginine/kg fish tissue, 4.82 mg cadaverine/kg fish tissue, 16.56 mg putrescine/kg fish tissue, 3.21 mg spermidine/kg fish tissue, 4.26 of redness, 8.17 of yellowness, 2.88 mg malondialdehyde/kg fish tissue, 9.44 nmol carbonyls/mg protein and 2092.58 g of hardness), respectively, on day 13 of storage when the control fillets were unfit for consumption (7 log CFU/g) (p < 0.05). However, in the same day of storage, both UV-C doses had similar values for BA (p > 0.05), higher amounts of FAA (44.28 and 44.13 mg L-lysine/kg fish tissue, 5.16 and 5.12 mg L- ornithine/kg fish tissue, 40.20 and 40.28 mg L-arginine/kg fish tissue), redness (4.86 and 5.33), yellowness (9.32 and 10.01), lipid oxidation (3.09 and 3.52 mg malondialdehyde/kg fish tissue) and protein oxidation (10.27 and 11.93 nmol carbonyls/mg protein), as well as lower hardness (1877.54 and 1767.39 g), respectively, than control fillets (p < 0.05). The combined preservation methods were the most effective in extending the shelf life and prolonging the physicochemical quality of the refrigerated tilapia fillets and the O₂ scavenger proved to be a potential alternative to prevent the negative changes induced by both UV-C doses.

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Fish is rich in nutrients, but is highly perishable due to rapid endogenous enzyme and bacterial activity in the postmortem period, resulting in the production of undesirable metabolites (e.g., total volatile basic nitrogen, ammonia and biogenic amines), limited shelf life and loss of quality. According to the United Nations Food and Agriculture Organization, approximately 27% of the fish catch is discarded because of loss of quality between capture and final consumption, leading to economic loss. Nile tilapia (Oreochromis niloticus) is the most important freshwater fish species contributing to the increase in global production and consumption of fish from aquaculture systems. Tilapia is usually consumed as fillets, which contain high amounts of protein (23%) and unsaturated fatty acids (66%), making the flesh more susceptible to protein and lipid oxidation. Previous studies have suggested a relationship between lipid and protein oxidation, wherein protein oxidation is favored by secondary compounds from lipid oxidation, and the free iron from protein oxidation catalyzes the lipid oxidation, causing changes in the color and texture and accelerating deterioration.

Vacuum packaging (VP) and modified-atmosphere packaging (MAP) are widely used for fish flesh, to minimize oxygen-induced reactions and to inhibit the growth of obligate aerobic microorganisms; however, these packaging systems require costly equipment and do not prevent O₂ from penetrating through the packaging film during storage. O₂ scavengers or O₂ absorbers, which can prevent O₂ penetration, are commercially available in the form of sachets, labels, cards or films. Their mechanism of action is based mainly on iron oxidation, wherein ferrous oxide (Fe²⁺) is converted to ferric oxide (Fe³⁺), reducing O₂ levels in the package to less than 0.01%. O₂ scavengers do not require the use of equipment and, therefore, it may be an efficient and economical alternative to the use of VP and MAP. Additionally, the effectiveness of the O₂ scavengers in increasing shelf life and preventing oxidative processes in fish species have been described in the literature.

UV-C radiation (wavelengths of 200–280 nm) is an emerging non-thermal technology that is effective in improving the bacterial quality and extending the shelf life of fish flesh through direct action on the microbial DNA, by formation of cross-linking between thymine and cytosine, and indirect action by water radiolysis, releasing free radicals. This technology has several advantages, including ease of implementation, low cost and absence of toxic residues. Previous studies confirmed that UV-C radiation is able to reduce the bacterial growth rate during refrigerated storage of fish species. However, in general, the UV-C doses needed to significantly extend the shelf life may lead to the production of reactive oxygen species (ROS), which remove a hydrogen atom from a weak C-H bond, consequently initiating a free-radical chain reaction and intensifying the oxidative processes, changes in texture and color during refrigerated storage. This effect depends mainly on the type and load of microorganisms present in the food matrix and the food composition and UV-C is therefore not necessarily dose-dependent. Deprivation of oxygen in the package during storage could minimize the adverse effects of UV-C radiation.

The demand for a longer shelf life while maintaining the physicochemical characteristics without the use of chemical preservatives has increased, and represents one of the main challenges for the food industry and scientific community. The number of studies on combined preservation methods has increased, but there is no report about the use of an O₂ scavenger in combination with UV-C radiation to treat any food matrix. Therefore, this study investigated the effect of an O₂ scavenger and two different doses of UV-C radiation (0.102 and 0.301 J/cm²), alone or in combination, on the quality attributes of Nile tilapia fillets stored at 4 ± 1 °C for 23 days.

Material and Methods

Experimental design. Twenty-five kilograms of fresh tilapia (Oreochromis niloticus) fillets packed in low-density polyethylene bags were purchased from a local fish farm in Rio de Janeiro, Brazil (22°27′46″S 042°39′10″W). Fillets (111.24 g ± 7.18 g each) were transported in ice chests to the laboratory, where they were individually packed in nylon/polyethylene bags (15 cm width, 22 cm height, 80 μm thickness) with barrier properties of 66.31 cc/m²/day for O₂ transmission rate (OTR) and 4.91 gm/m²/day for water-vapor transmission rate (WVTR) according to the information from the manufacturer (Gabrilina, São Paulo, Brazil). The fillets were randomly divided into six treatments according to packaging conditions (air or oxygen scavenger) and exposure to different UV-C doses (0.102 J/cm² or 0.301 J/cm²). The treatments were AP (air packaging), OSP (oxygen-scavenger packaging), AUV1 (air packaging + UV-C at 0.102 J/cm²), OSUV1 (oxygen-scavenger packaging + UV-C at 0.102 J/cm²), AUV3 (air packaging + UV-C at 0.301 J/cm²) and OSUV3 (oxygen-scavenger packaging + UV-C at 0.301 J/cm²). After the O₂ scavenger sachets were placed and the samples were radiated with UV-C, they were stored at 4 ± 1 °C for 23 days. All experiment was carried out in duplicate (n = 2).

Oxygen scavenger system. In the OSP, OSUV1 and OSUV3 treatments, an oxygen-scavenger sachet was placed inside the package before sealing. The sachet used was the Ageless SS-50, with O₂ absorption capacity of 50 mL (Mitsubishi Gas Chemical Co., Inc., Tokyo, Japan). This sachet reduces oxygen levels through spontaneous iron oxidation, converting ferrous oxide (Fe²⁺) to ferric oxide (Fe³⁺) in the presence of oxygen, resulting in an O₂ concentration <0.01% according to information from the manufacturer (Mitsubishi Gas Chemical Co., Inc., Tokyo, Japan).
**UV-C radiation exposure.** After packaging, AUV1, OSUV1, AUV3 and OSUV3 were subjected to UV-C radiation in an apparatus containing six 30-W lamps and six 55-W lamps (Osrám HNS, OFR, Munich, Germany) designed by Lázaro et al.\(^{20}\). The samples were placed in the center of the UV-C apparatus at a distance of 14 cm from the lamps. The intensity levels were monitored with a UV radiometer (MRUR-203, Instруtherm Ltda., São Paulo, Brazil) wrapped with the same sample packaging, and the exposure times were measured every 5 s until the doses of 0.102 ± 0.001 J/cm\(^2\) for AUV1 and OSUV1, and 0.301 ± 0.001 J/cm\(^2\) for AUV3 and OSUV3 were reached. These doses were chosen due to its effectiveness in increasing shelf life while causing physicochemical changes in refrigerated tilapia fillets conforming previously reported by some authors\(^{14,47}\).

**Bacterial analysis.** Serial dilutions were inoculated through the pour-plate technique into Petri dishes containing a plate-count agar (PCA, Merck, Darmstadt, Germany) for TAMC and TAPC, and Violet-Red-Bile-Glucose agar (VRBG-agar, Merck, Darmstadt, Germany) for Enterobacteriaceae, using a Spiral Plater (Eddy Jet 2, IUL Instruments, USA) mode E50. TAMC, TAPC and Enterobacteriaceae were enumerated in the electronic counter (Flash & Go, IUL Instruments, USA) after incubation at 37°C for 48 h, 10°C for 7 days, and 35°C for 24 h, respectively\(^{25}\). The results were expressed as log CFU/g fish tissue.

**Free amino acids analysis.** L-lysine, L-ornithine and L-arginine were analyzed as described by Gatti et al.\(^{26}\) with modifications in the sample deproteinization step. In brief, 0.1 g of sample (tissue) was mixed with 1 mL of 1.5 M perchloric acid (v/v) to remove proteins. After 2 min at room temperature, 0.325 mL H\(_2\)O and 0.5 mL potassium carbonate were added. The tubes were centrifuged at 10,000 × g for 2 min. The sample (50 µL) was mixed with 50 µL H\(_2\)O and 40 µL of 2.5-dimethyl-1H-pyrrole-3,4-dicarbaldehyde (DPD) reagent solution (v/v) for 10 min. 360 µL of the mobile phase (0.05 M triethylammonium phosphate buffer) was added to the derivatized solution, which was immediately analyzed by HPLC. The HPLC device was equipped with an ACE C18 3-µm reversed-phase column (250 × 4.6 mm I.D.), a 5-µm Ascentis C18 reversed-phase guard column (20 × 4.6 mm I.D.) and an RF-10AXL photodiode array detector (SHIMADZU, Kyoto, Japan), monitoring the absorbance at 320 nm. The results were expressed as mg free amino acids/kg fish tissue.

**Biogenic amines analysis.** Cadaverine, putrescine and spermidine were determined according to the method of Lázaro et al.\(^{25}\), using an HPLC (SHIMADZU, Kyoto, Japan) equipped with a CBM-20A controller composed of an LC-20AD pump, SPD-M20A diode-array detector, CT0-20A oven and SIL-20AC autosampler. The amines were separated using a Spherisorb ODS2 C18 column (15 × 0.46 cm I.D., 5 µm particle size) for the stationary phase, and an acetonitrile:water mixture (42:58, v/v) as the mobile phase, under isocratic conditions. The biogenic amines were detected at 198 nm, and the results were expressed as µg biogenic amines/kg fish tissue.

**Determination of total volatile basic nitrogen (TVB-N) and ammonia (NH\(_3\)).** TVB-N was determined by Conway’s microdiffusion method\(^{27}\) and the results were expressed as mg TVB-N/100 g fish tissue. Ammonia was quantified by the colorimetric method, using a UV-1800 spectrophotometer (SHIMADZU, Kyoto, Japan) at 425 nm according to the protocol of Rodrigues et al.\(^{11}\). Results were expressed as µg NH\(_3\)/g fish tissue, based on a standard curve (R\(^2\) = 0.996) constructed from seven different NH\(_3\) concentrations (1 to 15 µg NH\(_3\)).

**Determination of lipid and protein oxidation.** Lipid oxidation was evaluated by the thiobarbituric acid-reactive substances (TBARS) assay according to the method of Yin et al.\(^{16}\) adapted by Joseph et al.\(^{28}\). The absorbance values were read at 532 nm, using a UV-1800 spectrophotometer (SHIMADZU, Kyoto, Japan), and the results were expressed as mg malonaldehyde (MDA)/kg fish tissue from a standard curve (R\(^2\) = 0.999) constructed with eight different MDA concentrations (0.5 to 400 µmol). Protein oxidation was evaluated by the carbonyl content, following the method of Oliver et al.\(^{29}\) with modifications\(^{30}\). The absorbance values were measured at 280 nm (protein) and 370 nm (carbonyl) by a UV-1800 spectrophotometer (SHIMADZU, Kyoto, Japan), and the results were expressed as nmol carbonyls/mg protein. Protein content was determined by a standard curve (R\(^2\) = 0.999) constructed from five different concentrations of bovine serum albumin (0.1–1.0 mg), while the carbonyl content was calculated using an absorptivity coefficient for the protein hydrazones of 21.0/4.6 mm\(^2\)J/mole cm.

**Instrumental color measurements.** Lightness (L\(^*\)), redness (a\(^*\)) and yellowness (b\(^*\)) values were measured with an illuminant A, 8 mm-diameter aperture, and 10° standard observer through a Minolta CM-600D portable spectrophotometer (Minolta Camera Co., Osaka, Japan). The color parameters were determined at four random locations on the surface of each fillet immediately after it was removed from the packaging\(^{29}\).

**Instrumental texture profile.** The texture-profile analysis (TPA) was measured utilizing a TA.XTplus Texture Analyser (Stable Micro Systems, Surrey, UK) equipped with a cylindrical P/36 R probe. Each fillet was cut transversely into four pieces (2 × 2 × 2 cm\(^3\)), which were compressed twice to 50% of their original height with the time of 5 s between the two compression cycles, and pre-test, test speed, and post-test of 1 mm/s following conditions established by Sun et al.\(^{32}\). The parameters determined were hardness, chewiness, cohesiveness, springiness, and resilience.

**Statistical analysis.** The experiment was conducted in duplicate, using a fully randomized design (n = 2). A linear regression analysis was performed separately for each treatment to investigate the relationship between each physicochemical parameter and days of storage. The area under the curve (AUC), calculated by the trapezoidal method, was used to calculate the total amount of each physicochemical parameter produced during a time interval. To identify differences in the AUC among treatments (AP, OSP, AUV1, OSUV1, AUV3 and OSUV3), a one-way ANOVA was used. An additional post-hoc test with Tukey’s adjustment was performed. All analyses
Table 1. Bacterial growth parameters of tilapia (Oreochromis niloticus) fillets non- and treated with oxygen scavenger packaging (OSP) and ultraviolet radiation (UV-C) stored at 4 ± 1 °C for 23 days. Results are expressed as means ± standard deviation (n = 2). Different letters in the same row indicate significant differences (p < 0.05) among treatments. TAMC - Total aerobic mesophilic count; TAPC - Total aerobic psychrotrophic count. Lag - lag phase (h); EGR - exponential growth rate (log CFU/g/h); NC - number of colonies in the stationary phase (log CFU/g).

| Microorganisms | Parameters | Treatments | AP | OSP | AUV1 | OSUV1 | AUV3 | OSUV3 |
|----------------|------------|------------|----|-----|-----|-------|-----|-------|
| TAMC           | Lag        | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |
|                | EGR        | 0.45 ± 0.01 | 0.24 ± 0.01 | 0.21 ± 0.01 | 0.12 ± 0.01 | 0.20 ± 0.01 |
|                | NC         | 7.35 ± 0.01 | 7.86 ± 0.02 | 7.54 ± 0.34 | 7.72 ± 0.02 | 7.70 ± 0.00 |
| TAPC           | Lag        | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |
|                | EGR        | 0.48 ± 0.01 | 0.30 ± 0.01 | 0.38 ± 0.03 | 0.24 ± 0.01 | 0.37 ± 0.01 |
|                | NC         | 7.87 ± 0.14 | 7.85 ± 0.00 | 8.24 ± 0.01 | 7.80 ± 0.02 | 8.22 ± 0.02 |
| Enterobacteriaceae | Lag     | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |
|                | EGR        | 0.60 ± 0.00 | 0.30 ± 0.01 | 0.31 ± 0.01 | 0.23 ± 0.00 | 0.30 ± 0.01 |
|                | NC         | 6.63 ± 0.17 | 7.76 ± 0.04 | 7.80 ± 0.00 | 7.70 ± 0.01 | 7.72 ± 0.04 |

Figure 1. Total aerobic mesophilic count (a), Total aerobic psychrotrophic count (b), and Enterobacteriaceae count (c) in tilapia (Oreochromis niloticus) fillets non- and treated with oxygen scavenger packaging (OSP) and ultraviolet radiation (UV-C) stored at 4 ± 1 °C for 23 days. Results are expressed as the mean of log CFU (colony forming units)/g ± standard deviation (n = 2). AP (air packaging); OSP (oxygen scavenger packaging); AUV1 (air packaging + UV-C at 0.102 J/cm²); OSUV1 (oxygen scavenger packaging + UV-C at 0.102 J/cm²); AUV3 (air packaging + UV-C at 0.301 J/cm²); and OSUV3 (oxygen scavenger packaging + UV-C at 0.301 J/cm²).

Results and Discussion

Bacterial growth during storage. The results for TAMC, TAPC and Enterobacteriaceae are shown in Table 1 and Fig. 1a–c. The lag phase was absent in all bacterial groups. Although the number of colonies in the stationary phase of the fillets treated with the oxygen scavenger and/or UV-C radiation (0.102 or 0.301 J/cm²) was higher than in the fillets in air packaging (AP), these emerging techniques alone or in combination extended the...
shelf life of the tilapia fillets by decreasing (p < 0.05) the exponential growth rate (EGR) of the microorganisms (Table 1). The initial bacterial counts were 4.24 log CFU/g for TACM, 3.45 log CFU/g for TAPC and 2.78 log CFU/g for Enterobacteriaceae. Considering the limit of 3 log CFU/g for initial counts of Enterobacteriaceae proposed by the International Commission on Microbiological Specifications for Foods (ICMSF)33, the tilapia fillets showed good initial microbial quality. The limit of 7 log CFU/g for TACM proposed by ICMSF33 was also used as the microbiological criterion to establish the shelf life of tilapia fillets during refrigerated storage. AP exceeded the limit of 7.0 log CFU/g for TACM on day 9, while OSP, AUV1, AUV3, OSUV1 and OSUV3 reached this limit on storage days 14, 15, 16 and 16, respectively.

The microbiota of tropical freshwater fishes such as tilapia is composed predominantly of Gram-negative aerobic and facultative anaerobic bacteria, including bacteria from the family Enterobacteriaceae, and Gram-positive bacteria34. Molinari et al.35 and Pakingking et al.36, evaluating the microbiota of tilapia, found a wide variety of bacterial genera and species, including Pseudomonas spp., Shewanella putrefaciens, Aeromonas spp., Pasteurella pneumotropica, Photobacterium damselae, Plesiomonas shigelloides, Vibrio spp., Burkholderia cepacia, Chromobacterium violaceum, and Flavimonas oryzihabitans (Gram-negative aerobic and facultative anaerobic bacteria); Citrobacter spp., Edwardsiella spp., Enterobacter cloacae, Klebsiella oxytoca, Escherichia coli (Enterobacteriaceae); and Bacillus sp. and Staphylococcus sp. (Gram-positive bacteria). However, along with the increase in the storage time under aerobic conditions, Pseudomonas spp. became the dominant spoilage bacteria in refrigerated fish, due to low temperature34.

In our study, the oxygen scavenger and both UV-C doses (OSP, AUV1 and AUV3) had similar effects on EGR for TACM and Enterobacteriaceae. However, UV-C radiation (AUV1 and AUV3) showed a higher (p < 0.05) EGR for TAPC than OSP. This fact may be explained by antimicrobial effect of the UV-C16,17. Gram-negative bacteria are more sensitive to UV-C radiation due to the lack of a thick cell wall, which prevents UV-C absorption by microbial DNA37. Nevertheless, although Pseudomonas spp. are Gram-negative, they are resistant to radiation due to their ability to form a biofilm in response to UV-C induced stress, in an attempt to repair damaged DNA38,39. On the other hand, obligate aerobic bacteria such as Pseudomonas spp. are highly sensitive to low oxygen concentrations from O2 scavenger13. Our results demonstrated that the O2 scavenger delayed the EGR of Enterobacteriaceae, which are facultative anaerobic bacteria. This delay can be attributed to the sensitivity of these bacteria to carbon dioxide, which increases in the package headspace due to the relative decrease in the O2 level caused by O2 scavengers13,40.

With respect to the combined preservation methods, the oxygen scavenger plus UV-C radiation, at both doses (OSUV1 and OSUV3), was the most effective in delaying the EGR in all bacterial groups, indicating a synergistic effect between the two preservation methods. While the O2 scavenger inhibits the growth of obligate aerobic bacteria and decreases the growth rate of facultative anaerobic bacteria of family Enterobacteriaceae by removing O2 and increasing the CO2 level inside the package, UV-C radiation decreases the growth rate of the microorganisms, especially Gram-negative bacteria, through direct or indirect damage to microbial DNA13,16,17.

OSP, AUV1, OSUV1, AUV3 and OSUV3 showed more viable cells in the stationary phase than AP. This difference may be explained by sublethal injury induced by CO2 and UV-C radiation to bacterial cells, which at first grow more slowly than intact cells, and more rapidly after recovery, mainly in an environment without natural competition13,41,42.

In agreement with the present results, previous researchers demonstrated that O2 scavengers were effective in extending the shelf life of refrigerated trout-fillets14 and ground beef15 by 5 and 2 days, respectively. Mohan et al.16 found an extension of 6–7 days in the shelf life of sardines packed with an O2 scavenger. Likewise, Bottino et al.17 reported that UV-C at 0.055 and 0.160 J/cm2 extended the shelf life of tambacu (Colossoma macropomum × Piaractus mesopotamicus) fillets stored at 4 °C by 50% and 100%, respectively. Monteiro et al.18 observed that the shelf life of refrigerated tilapia fillets exposed to UV-C radiation at 0.103 J/cm2 was extended by at least 2.5-fold.

### Free amino acids and biogenic amines

The levels of free amino acids (L-lysine, L-ornithine, L-arginine) and biogenic amines (cadaverine, putrescine, spermidine) increased in all treatments throughout the storage period (p < 0.05; Table 2). AUV1 and AUV3 showed higher total amounts (p < 0.05), while OSP, OSUV1 and OSUV3 had lower (p < 0.05) total amounts of free amino acids than AP throughout the storage period (Table 2). The results of free amino acids and biogenic amines in all days of storage can be found as Supplementary Table S1.

The increase of free amino acids during storage is attributed to the action of endogenous and microbial proteolytic enzymes44. Our results are attributable to the resistance of Pseudomonas spp. to UV-C radiation, together with the effect of UV-C in increasing the amount of oxidized proteins, which are more susceptible to proteolysis, resulting in a high level of free amino acids17,36,39. On the other hand, oxygen scavenger is highly effective against Pseudomonas spp.13,40 and it is able to minimize ROS-induced oxidation65.

Regarding biogenic amines, cadaverine, putrescine and spermidine are formed mainly by bacterial decarboxylation of precursor free amino acids such as L-lysine, L-ornithine and L-arginine, respectively46. Metabolization of L-arginine to L-ornithine is another pathway to formation of putrescine46, which explains the high amount of this amine in relation to others (cadaverine and spermidine). The present study found no differences (p > 0.05) in the total amounts of cadaverine, putrescine and spermidine among AP, AUV1 and AUV3; whereas OSP, OSUV1 and OSUV3 resulted in lower (p < 0.05) total amounts of these biogenic amines than the other treatments (Table 2). Although O2, OSP, AUV1 and AUV3 had similar effect in controlling the growth of Enterobacteriaceae, which is the main bacterial group associated with the formation of biogenic amines37, UV-C radiation may cause oxidative decarboxylation of amino acids by catalyzing the production of Fe3+48,49. On the other hand, O2 absorber has the capacity to minimize the oxidative reaction pathways by oxygen scavenging45, explaining our results for combined preservation methods (OSUV1 and OSUV3).
Currently, there is little information about the effect of O₂ absorbers and UV-C radiation on the production of free amino acids and biogenic amines in fish species during refrigerated storage. Similarly to our results, an increase in the amount of free amino acids by UV-C has been previously reported in fish stored at 4 °C.11,19

**Table 2.** Free amino acids and biogenic amines of tilapia (*Oreochromis niloticus*) fillets non- and treated with oxygen scavenger packaging (OSP) and ultraviolet radiation (UV-C) stored at 4 ± 1 °C for 23 days. Results are expressed as means ± standard deviation (n = 2). a,b,c Different superscripts in the same column indicate significant differences (p < 0.05) among treatments. AUC – Area under curve; AUC0–13 – from day 0 to 13 among treatments AP, OSP, AUV1, OSUV1, AUV3, and OSUV3; AUC15–23 – from day 15 to 23 among treatments OSP, AUV1, OSUV1, AUV3, and OSUV3. NA – Not applicable. AP (air packaging); OSP (oxygen scavenger packaging); AUV (air packaging + UV-C at 0.102 J/cm²); OSUV (oxygen scavenger packaging + UV-C at 0.102 J/cm²); AUV3 (air packaging + UV-C at 0.301 J/cm²); and OSUV3 (oxygen scavenger packaging + UV-C at 0.301 J/cm²).

**Total volatile basic nitrogen (TVB-N) and ammonia (NH₃).** The initial levels of TVB-N and NH₃ were 10.08 ± 0.00 mg TVB-N/100 g and 7.66 ± 0.04 μg NH₃/g fish tissue. As expected, the TVB-N and ammonia levels increased (p < 0.05) in all treatments during the storage period, with the highest increases in the tilapia fillets under aerobic packaging (AP; Table 3). However, no treatment exceeded the limit of 25 mg TVB-N/100 g
established by the Commission of the European Community until the end of storage, indicating that N-TVB was not a good indicator of bacterial spoilage and quality loss in tilapia fillets stored under refrigeration. On days 9, 14, 15, 16 and 15 of refrigerated storage, when the acceptable microbial limit of 7 log CFU/g was reached, the TVB-N levels were 17.75 ± 0.89, 15.85 ± 0.07, 17.35 ± 0.81, 14.15 ± 0.27, 17.39 ± 0.10 and 14.09 ± 0.10 mg TVB-N/100 g for AP, OSP, AUV1, OSUV1, AUV3 and OSUV3, respectively (Supplementary Table S2). In freshwater fish species, TVB-N values are related mainly to the ammonia concentration, due to absence or low level of trimethylamine oxide in vivo. However, there is no limit for ammonia content in freshwater fish species. In the present study, at the point when the fillets were unfit for consumption (7 log CFU/g), the ammonia levels were 17.75 ± 0.89, 15.85 ± 0.07, 17.35 ± 0.81, 14.15 ± 0.27, 17.39 ± 0.10 and 14.09 ± 0.10 mg ammonia/g fish tissue for AP, OSP, AUV1, OSUV1, AUV3 and OSUV3, respectively (Supplementary Table S2). In fresh-water fish species, TVB-N values are related mainly to the ammonia concentration, due to absence or low level of trimethylamine oxide in vivo.

### Table 3. Physicochemical parameters of tilapia (Oreochromis niloticus) fillets non- and treated with oxygen scavenger packaging (OSP) and ultraviolet radiation (UV-C) stored at 4 ± 1°C for 23 days. Results are expressed as means ± standard deviation (n = 2).

| Parameters (mg TVB-N/100 g fish tissue) | Treatments | AUC<sup>3</sup> | Linear regression coefficients | y-intercept | slope | p-value | r-squared |
|----------------------------------------|-------------|------------------|--------------------------------|-------------|--------|---------|-----------|
|                                        |             | AUC<sub>1-15</sub> | AUC<sub>15-23</sub> |                           |         |         |           |
| Ammonia (µg NH₃/g fish tissue)          | AP          | 129.80 ± 0.33<sup>a</sup> | NA | 7.44 ± 0.09 | 0.40 ± 0.01 | <0.0001 | 0.981 |
|                                        | OSP         | 117.90 ± 0.22<sup>b</sup> | 90.33 ± 0.22<sup>c</sup> | 7.65 ± 0.08 | 0.20 ± 0.01 | <0.0001 | 0.970 |
|                                        | AUV1        | 122.80 ± 0.33<sup>d</sup> | 94.43 ± 0.20<sup>e</sup> | 7.88 ± 0.11 | 0.21 ± 0.01 | <0.0001 | 0.953 |
|                                        | OSUV1       | 109.70 ± 0.19<sup>f</sup> | 84.01 ± 0.16<sup>g</sup> | 7.40 ± 0.04 | 0.16 ± 0.00 | <0.0001 | 0.991 |
|                                        | AUV3        | 122.90 ± 0.32<sup>h</sup> | 94.47 ± 0.24<sup>i</sup> | 7.88 ± 0.12 | 0.21 ± 0.01 | <0.0001 | 0.949 |
|                                        | OSUV3       | 109.80 ± 0.35<sup>j</sup> | 84.16 ± 0.21<sup>k</sup> | 7.40 ± 0.04 | 0.16 ± 0.00 | <0.0001 | 0.991 |
| TVB-N (mg TVB-N/100 g fish tissue)     | AP          | 216.50 ± 0.99<sup>l</sup> | NA | 119.98 ± 0.60 | 0.72 ± 0.09 | <0.0001 | 0.788 |
|                                        | OSP         | 178.50 ± 4.24<sup>m</sup> | 152.60 ± 3.34<sup>n</sup> | 10.60 ± 0.31 | 0.45 ± 0.02 | <0.0001 | 0.923 |
|                                        | AUV1        | 192.00 ± 4.94<sup>o</sup> | 170.80 ± 3.04<sup>p</sup> | 11.11 ± 0.35 | 0.54 ± 0.03 | <0.0001 | 0.930 |
|                                        | OSUV1       | 154.00 ± 7.20<sup>q</sup> | 125.60 ± 3.76<sup>r</sup> | 9.86 ± 0.20 | 0.31 ± 0.02 | <0.0001 | 0.928 |
|                                        | AUV3        | 191.70 ± 3.31<sup>s</sup> | 171.10 ± 1.27<sup>t</sup> | 11.08 ± 0.34 | 0.54 ± 0.03 | <0.0001 | 0.932 |
|                                        | OSUV3       | 154.20 ± 3.70<sup>u</sup> | 125.70 ± 1.73<sup>v</sup> | 9.87 ± 0.18 | 0.31 ± 0.01 | <0.0001 | 0.942 |
| Lipid oxidation (mg malonaldehyde/kg fish tissue) | AP          | 19.46 ± 0.20<sup>w</sup> | NA | 0.03 ± 0.00 | 0.22 ± 0.00 | <0.0001 | 0.963 |
|                                        | OSP         | 9.84 ± 0.09<sup>x</sup> | 20.91 ± 0.12<sup>y</sup> | 0.09 ± 0.01 | 0.14 ± 0.01 | <0.0001 | 0.960 |
|                                        | AUV1        | 23.13 ± 0.18<sup>z</sup> | 28.88 ± 0.14<sup>aa</sup> | 0.49 ± 0.01 | 0.17 ± 0.01 | <0.0001 | 0.934 |
|                                        | OSUV1       | 10.11 ± 0.11<sup>ab</sup> | 21.05 ± 0.20<sup>ac</sup> | 0.07 ± 0.00 | 0.14 ± 0.01 | <0.0001 | 0.960 |
|                                        | AUV3        | 27.99 ± 0.30<sup>ad</sup> | 32.70 ± 0.10<sup>ae</sup> | 0.76 ± 0.01 | 0.18 ± 0.01 | <0.0001 | 0.928 |
|                                        | OSUV3       | 10.36 ± 0.10<sup>af</sup> | 21.07 ± 0.29<sup>ag</sup> | 0.04 ± 0.00 | 0.14 ± 0.01 | <0.0001 | 0.963 |
| Protein oxidation (nmol carbonyl/mg protein) | AP          | 71.08 ± 1.67<sup>ah</sup> | NA | 1.68 ± 0.16 | 0.58 ± 0.02 | <0.0001 | 0.971 |
|                                        | OSP         | 43.78 ± 1.36<sup>ai</sup> | 48.35 ± 0.83<sup>aj</sup> | 1.71 ± 0.12 | 0.23 ± 0.01 | <0.0001 | 0.951 |
|                                        | AUV1        | 88.09 ± 1.67<sup>ak</sup> | 97.15 ± 2.35<sup>al</sup> | 3.60 ± 0.26 | 0.46 ± 0.02 | <0.0001 | 0.944 |
|                                        | OSUV1       | 44.04 ± 1.85<sup>am</sup> | 48.78 ± 0.62<sup>an</sup> | 1.74 ± 0.13 | 0.23 ± 0.01 | <0.0001 | 0.948 |
|                                        | AUV3        | 98.12 ± 2.65<sup>ao</sup> | 117.20 ± 4.80<sup>ap</sup> | 3.76 ± 0.29 | 0.58 ± 0.02 | <0.0001 | 0.956 |
|                                        | OSUV3       | 44.71 ± 2.55<sup>aq</sup> | 48.69 ± 1.10<sup>ar</sup> | 1.80 ± 0.14 | 0.23 ± 0.01 | <0.0001 | 0.941 |

**Lipid and protein oxidation.** An increase in the malonaldehyde (MDA) and carbonyl levels was observed during refrigerated storage in all treatments, especially in AUV1 and AUV3 (Table 3). The increases in lipid and protein oxidation by UV-C radiation were dose-dependent. AUV3 showed the highest (p < 0.05) MDA and carbonyl levels during the storage period, followed by AUV1, AP, and treatments with the O₃ scavenger (OSP, OSUV1 and OSUV3), which did not differ from each other (p > 0.05; Table 3).

A concomitant lipid and protein oxidation has been described in literature and it was also observed in our study. Lipid and protein oxidation occur mainly in the presence of reactive oxygen species (ROS). Therefore,
Table 4. Instrumental color parameters of tilapia (Oreochromis niloticus) fillets non- and treated with oxygen scavenger packaging (OSP) and ultraviolet radiation (UV-C) stored at 4 ± 1 °C for 23 days. Results are expressed as means ± standard deviation (n = 2). *Different superscripts in the same column indicate significant differences (p < 0.05) among treatments. †AUC – Area under curve; AUC0-13 – from day 0 to 13 among treatments AP, OSP, AUV1, OSUV1, AUV3, and OSUV3; AUC15-23 – from day 15 to 23 among treatments OSP, AUV1, OSUV1, AUV3, and OSUV3. NA – Not applicable. AP (air packaging); OSP (oxygen scavenger packaging); AUV1 (air packaging + UV-C at 0.102 J/cm²); OSUV1, AUV3, and OSUV3 (oxyg
Instrumental texture parameters. Hardness, chewiness, cohesiveness, springiness and resilience decreased (p < 0.05) during the refrigerated period in all treatments (Table 5). OSP, OSUV1 and OSUV3 showed the highest (p < 0.05) hardness and chewiness, followed by samples submitted to air packaging (AP) and UV-C radiation at both doses (AUV1 and AUV3) during the storage period (Table 5). Cohesiveness, springiness and resilience were not affected (p > 0.05) by the O2 absorber and/or UV-C radiation, regardless of the dose, during the refrigerated storage period. The results of instrumental texture parameters in all days of storage can be found as Supplementary Table S4.

Softening during the post-mortem period is related to the activity of endogenous and microbial proteolytic enzymes, which results in protein breakdown. The results for hardness and chewiness in this study can be explained by the results for free amino acids, MDA level, carbonyl content, and TAPC. The pro-oxidant effect of the UV-C radiation increased the amount of free amino acids, indicating a higher proteolysis rate, while ROS formation at 0.102 and 0.301 J/cm² was mitigated by O2 absorber. Furthermore, when compared to OSP, OSUV1 and OSUV3, both UV-C doses were less effective against growth of aerobic psychrotrophic bacteria, where Pseudomonas spp. is the dominant proteolytic spoilage bacteria in freshwater fish species. There are no studies related to instrumental texture parameters in fish species packed with an O2 scavenger. However, in agreement with previous studies, Chounou et al. reported that an O2 absorber was effective in preventing discoloration in ground meat stored under refrigeration.

### Table 5. Instrumental texture parameters of tilapia (Oreochromis niloticus) fillets non- and treated with oxygen scavenger packaging (OSP) and ultraviolet radiation (UV-C) stored at 4 ± 1°C for 23 days. Results are expressed as means ± standard deviation (n = 2). a,b,c,dDifferent superscripts in the same column indicate significant differences (p < 0.05) among treatments. AUC – Area under curve; AUC_{0-13} – from day 0 to 13 among treatments AP, OSP, AUV1, OSUV1, AUV3, and OSUV3; AUC_{15-23} – from day 15 to 23 among treatments OSP, AUV1, OSUV1, AUV3, and OSUV3. NA – Not applicable. AP (air packaging); OSP (oxygen scavenger packaging); AUV1 (air packaging + UV-C at 0.102 J/cm²); OSUV1 (oxygen scavenger packaging + UV-C at 0.102 J/cm²); AUV3 (air packaging + UV-C at 0.301 J/cm²); and OSUV3 (oxygen scavenger packaging + UV-C at 0.301 J/cm²).
with our study, Monteiro et al. reported that a similar UV-C dose decreased the hardness and chewiness but did not affect the cohesiveness and springiness of tilapia fillets stored under refrigeration. Molina et al. also observed that UV-C treatment increased collagen degradation in sea bass fillets.

**Conclusion**

The O2 scavenger, both UV-C doses (0.102 and 0.301 J/cm²) and combinations of these preservation methods, independently of the radiation dose, retarded the bacterial growth and the formation of TVB-N and ammonia, increasing the shelf life of refrigerated tilapia fillets by more than 50%, 60% and 70%, respectively. While UV-C doses induced adverse changes in the color, texture and oxidative processes, O2 scavenger demonstrated to be an effective and simple alternative to reduce the negative effects of UV-C radiation. Therefore, the O2 scavenger combined with UV-C radiation, regardless of the dose (0.102 or 0.301 J/cm²), was the most effective method to extend the shelf life and retard the loss of physicochemical quality of tilapia fillets stored under refrigeration.

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Author contributions
M.I.G.M., E.T.M. and C.A.C.J. designed the experiment. M.I.G.M., Y.S.M., V.S.C., R.V.B.P.M. and T.S.A. performed the experiments. M.I.G.M. wrote the main manuscript text. All authors revised the final manuscript.

Competing interests
The authors declare no competing interests.

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