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Influence of vacuum and modified atmosphere packaging in combination with UV-C radiation on the shelf life of rainbow trout (*Oncorhynchus mykiss*) fillets

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ABSTRACT

The effects of UV-C radiation, modified atmosphere packaging (MAP) and their combination on rainbow trout (*Oncorhynchus mykiss*) fillets quality were examined during a period of 22 days. The samples were submitted to five packaging conditions: (AP) aerobic packaging; (VP) vacuum packaging; (VP + UV-C) vacuum packaging + UV-C radiation; (MAP) modified atmosphere packaging (80% CO_2/20% N_2) and (MAP + UV-C) modified atmosphere packaging + UV-C radiation (80% CO_2/20% N_2; 106.32 mJ/cm^2) and storaged at 4 °C. The samples were analyzed daily for microbiological (mesophilic, psychrotrophic and Enterobacteriaceae counts) and chemical (pH, TMA-N, TBV-N, lipid oxidation, ammonia and biogenic amines) parameters. Overall, UV-C radiation promoted lag phase formation in mesophilic and psychrotrophic groups. Mesophilic and psychrotrophic groups presented significant lower ($P < 0.05$) growth rate and colony forming units in the stationary phase in the samples submitted to MAP. MAP significantly reduced ($P < 0.05$) total mesophilic count and MAP + UV-C total mesophilic and psychrotrophic counts. Values of pH decreased at all packaging conditions except in the case of aerobic packaging. TBARS value increased faster in samples subject to MAP and MAP + UV-C whereas TMA-N, TVB-N and ammonia values increased slowly. Lower production of putrescine and cadaverine was observed in MAP and VP samples. MAP reduced total production of ammonia, TVB-N and putrescine, whereas MAP + UV-C reduced total production of TVB-N and cadaverine during entire the storage time. Results of the current study suggest that MAP retarded microbial growth and delayed chemical changes enhancing the shelf life of rainbow trout fillets at least twice.

1. Introduction

Fresh fish is considered one of the most perishable foods due to the strong action of autolytic enzymes and microbial activity resulting in changes in biological composition of matrix and rapid spoilage (Ashie, Smith, & Simpson, 1996; Borges, Conte-Junior, Franco, & Freitas, 2013; Gram & Huss, 1996; Rodrigues et al., 2013). Psychotropic microorganisms, such *Pseudomonas* spp., and Enterobacteriacea are some of the main spoilage microorganisms closely related to refrigerated fish deterioration and are incriminated in the production of several metabolites, which lead to quality loss and decreased the shelf-life of this product (Gram & Dalgaard, 2002; Gram & Huss, 1996; Rodrigues et al., 2012). In order to control microbial action and extend the shelf-life of several food matrices preservation technologies, such as modified atmosphere packaging (MAP) and short-wave ultraviolet (UV-C) radiation, have been studied (Chun, Kim, Lee, Yu, & Song, 2010; Lazaro et al., 2014; Milne & Powell, 2014; Monteiro et al., 2013; Shariat, Raftari, & Abu Bakar, 2013).

MAP and vacuum packaging are considered established technologies for food preservation and works respectively by changing the gas proportions in a food environment by withdrawing O_2 or

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replacing the atmosphere inside the package by a mixture of gases, such as carbon dioxide (CO_2) and nitrogen (N_2) gas (Nettles Cutter, 2002). CO_2 is the main gas used as bacteriostatic agent on fish microflora (Alfaro, Hernández, Le Marc, & Pin, 2013; Provincial, Guillén, Gil, et al., 2013; Turan & Kocatepe, 2013). Several studies have demonstrated that high CO_2 levels extend the shelf-life of fish products due to the inhibition of microbial growth (Arashisar, Hisar, Kaya, & Yanik, 2004; Campus et al., 2011; Özogul, Polat, & Özogul, 2004; Provincial, Guillén, Alonso, et al., 2013, Provincial, Guillén, Gil, et al., 2013). In addition, oxygen gas from the pack is substituted by nitrogen, an inert gas, resulting in inhibition of the growth of aerobic microorganisms and in decreasing the oxidative rancidity (Sivertsvik, Jeksrud, & Rosnes, 2002). The action of both technologies inhibits the normal spoilage-related microbiota and extends the shelf-life at cold temperatures, keeping the quality of the product (Provincial, Guillén, Gil, et al., 2013; Simões et al., 2014; Sivertsvik et al., 2002).

UV-C radiation is a technology used in food processing to improve the safety and extend the shelf life of food products by inactivating pathogenic and spoilage-related microorganisms on food surfaces (Guerrero-Beltrán & Barbosa-Cánovas, 2004). The wavelength range to obtain a germicidal effect is 220–300 nm, for which the maximum effect can be found at a wavelength around 260 nm, as approved by the Food and Drug Administration (FDA) for use in food products (Bintsis, Litopoulou-Tzanetaki, & Robinson, 2000; US FDA, 2007). The germicidal effect is attributed to injuries in the DNA of microorganisms caused by the absorption of the UV-C radiation leading to disruption of cell growth (Koutchma, Forney, & Moraru, 2009). This non-thermal technology presents several advantages for food industries, including easy deployment, low cost, and no chemical or radioactive residues. It also has an insignificant effect on the nutritional and sensory qualities of the products (Chun, Kim, Chung, Won, & Song, 2009). Numerous studies have demonstrated the action of UV-C radiation on the inactivation of pathogenic microorganisms on several food matrices (Chun, Kim, & Song, 2010, 2009; Haughton et al., 2011; Unluturk, Atilgan, Baysal, & Tari, 2008); however, the application of such technology on the surface of fish is still scarce.

Due to the fact that there is a lack of information about the use of UV-C radiation in fish packaged with MAP, the present study was undertaken using microbiological and physico–chemical parameters to evaluate the effect of these technologies, applied individually and combined, on the shelf life of rainbow trout (*Oncorhynchus mykiss*) fillets stored under refrigeration (4 °C).

2. Material and methods

2.1. Experimental design

Forty kilograms of eviscerated and decapitated rainbow trout were obtained from Trutas da Serrinha aquaculture farm in Itatiaia, Rio de Janeiro, Brazil. The fishes were harvested and slaughtered by hypothermic shock after immersion in ice-cold water. The samples were eviscerated, decapitated, packaged in ice (0 °C) and transported in a styrofoam box to the laboratory within three hours. The trout was filleted under sterile conditions. A total of 330 packages containing 400 g trout fillets were assembled and divided into 22 sets to undergo the following packaging conditions: (AP) aerobic packaging (with the presence of air); (VP) vacuum packaging (without the presence of air – removal the air by vacuum machine); (VP + UV-C) vacuum packaging + UV-C radiation (without the presence of air and submitted to UV-C radiation); (MAP) modified atmosphere packaging (without the presence of air and submitted to modified atmosphere packaging – MAP); (MAP + UV-C) modified atmosphere packaging + UV-C radiation (without the presence of air and submitted to UV-C radiation and MAP). All samples were aseptically packaged in low-density polyethylene gas-impermeable bags (20 cm × 30 cm) with diffusion coefficients, according to the supplier, of 150 cm^3/24 h m^2 bar of CO_2, 35 cm^3/24 h m^2 bar of O_2, and 1.4 cm^3/24 h m^2 bar of N_2 at 22 °C.

A vacuum-packaging machine (TECMAQ, Vacuum sealer, AP 450) was used to perform both modified atmosphere and vacuum packaging. The gas mixture used in MAP and MAP + UV-C was 80% CO_2 and 20% N_2, respectively, since previous studies reported that high concentrations of CO_2 can have positive effect on the inhibition of microbial growth (Bouletis et al., 2013; Provincial, Guillén, Alonso, et al., 2013, Provincial, Guillén, Gil, et al., 2013; Yew et al., 2014). A previously constructed stainless steel barrel-shaped chamber (Lazaro et al., 2014) with twelve UV-C lamps (six 30 W lamps and six 55 W lamps; low pressure mercury lamps with maximum emission peak of 253.7 nm; OSRAM™ HNS, OFR, Munich, Germany) distributed in interspersed positions was used for UV-C treatment. Before packaging, an intensity of 1.772 mW/cm^2 (determined by UV radiometer (MRUR-203™, Instrutherm, São Paulo, Brazil)) was applied on the surface of the rainbow trout fillets for 60 s, resulting in a UV-C dose of 106.32 mJ/cm^2.

After packaging, the samples were stored under refrigeration (4 °C) and were analyzed daily until the 22nd day of storage. All analyses were performed in experimental triplicate.

2.2. Microbiological analysis

Samples were taken to determine total aerobic mesophilic (TAMC) and psychrotrophic (TAPC) counts as well as Enterobacteriaceae count from each package, for each of the five packaging conditions performed. For such analysis, an amount of 25 g was removed aseptically, inserted in a sterile bag and homogenized with 225 mL of peptone water (0.1% sterile peptone, w/v) for 60 s in a stomacher (Stomacher 80, Seward, London, UK). Decimal dilutions were performed and 1 mL was removed from each dilution and cultured by the pour plate technique on plate count agar (PCA, Merck) for mesophilic and psychrotorophic counts and by the pour plate technique in Violet-Red-Bile-Glucose agar (VRBG-agar, Merck) plates for Enterobacteriaceae count. The PCA plates were incubated at 37 °C for 48 h for mesophilic count and at 7 °C for 10 days for psychrotrophic count. The VRBG plates were incubated at 37 °C for 48 h. Colony forming units were counted and expressed as log cfu g^{-1} (Isohanni & Lyhs, 2009).

2.3. Chemical analysis

2.3.1. Proximate analysis

Moisture was determined in fresh and untreated samples (raw material) by the Association of Official Analytical Chemist (AOAC, 2012) method. The lipid content of the fish was determined by an extraction performed by petroleum ether (AOAC, 2012). Protein content was determined by the micro Kjeldahl method (AOAC, 2012). The ash content was determined by total carbonization of the sample using a Bunsen burner with subsequent retention a muffle furnace at 550° C for 3 h to incinerate the sample until it was free of carbon particles.

2.3.2. pH

pH value was measured according the potentiometric method of Conte-Júnior, Souza, Baptista, Mársico, and Mano (2010), after homogenization of 10 g fish muscle samples in 90 mL distilled water.

2.3.3. Lipid oxidation

Lipid oxidation, measured as 2-thiobarbituric acid reactives substance (TBARS), was conducted using the method of Tarladgis,

Watts, Younathan, and Dugan (1960) and adapted by Monteiro et al. (2012). Briefly, 10 g of sample was added to 97.5 mL of distilled water and 2.5 mL of HCl (4 N) followed by homogenization and distillation. Subsequently, 5 mL of the distillate was added to 5 mL of 0.02 M TBA solution, heated in a water bath at 100 °C for 35 min and cooled. The measurement was performed immediately on a Smartspec Plus spectrophotometer (Bio-Rad Laboratories, São Paulo, Brazil) at a wavelength of 528 nm. Results were expressed in mg malondialdehyde (MA)/Kg of sample.

2.3.4. Trimethylamine (TMA-N) and total volatile bases (TVB-N)

TMA-N and TVB-N was determined by vapor distillation through the microdiffusion method of Conway and Byrne (1933). Results were expressed as mg TMA-N/100 g and mg TVB-N/100 g, respectively.

2.3.5. Ammonia

The quantitative determination of ammonia was adapted by the colorimetric method of McCullough (1967). In brief, 1 g of sample was added to 10 mL of Milli-Q water and homogenized for 30 s in a tube. After decanting, 1 mL of the supernatant was removed and 2 mL of Nessler's reagent was added. Next, the tube was homogenized for 30 s and the measurement was performed immediately on a SmartSpecTM Plus spectrophotometer (Bio-Rad Laboratories, São Paulo, Brazil) at a wavelength of 425 nm.

A calibration curve was performed after reading seven different concentrations (1, 2.5, 5, 7.5, 10, 15 and 20 μg NH_3/g) of a standard solution of ammonium sulphate (2 μg/ml). Each concentration was added in a 10 mL tube containing 1 mL of tungstate. One mL of 1 N sulphuric acid was added from a quick delivery pipette and the contents of the tube were mixed thoroughly. The tube was centrifuged at 503 × *g* for 10 min and the protein-free supernatant removed. 0.5 mL of clear supernatant was pipetted into a 10 mL tube and 2 mL of Nessler's reagent was added. The tube was homogenized for 30 s and the measurement was performed immediately on the spectrophotometer (SmartSpecTM Plus, Bio-Rad Laboratories, São Paulo, Brazil) at wavelength of 425 nm. The regression equation and the regression coefficient (r^2) were calculated from the linear calibration curve previously prepared and the results were expressed as μg NH_3/g.

2.3.6. Biogenic amines

The extraction and derivatization processes of samples were performed according to Lázaro et al. (2013). Briefly, the fish samples were extracted by perchloric acid (5%), neutralized by 2 N sodium hydroxide (reach pH > 12) and derivatized by addition of benzoyl chloride. Thereafter, diethyl ether was added to extract the mixture and this was evaporated in a stream of nitrogen. The residue was resuspended with 1000 μL of mobile phase.

A chromatographic analysis to putrescine and cadaverine determination was carried out using a Shimadzu Prominence UFLC apparatus (Shimadzu, Kyoto, Japan) equipped with a Water Spherisorb ODS-2 C18 (15 × 0.46 cm id., 5 μm) column, a Supelco guard column, Ascentis C18 (2 × 0.40 cm id., 5 μm) and a diode array detector (SPD-M20A). All analyses were performed in isocratic condition.

2.3.7. Free amino acids analysis

Analyses of untreated samples (raw material) were carried out to determine the initial concentration of free amino acids L-lysine, L-ornitine and L-arginine. The free amino acids analyses were performed according to Alvares, Conte-Junior, Silva, and Paschoalin (2012). In brief, the frozen tissue sample was deproteinized by perchloric acid (1.5 M). 6 mL of ultrapure water and 500 μL of K_2CO_3 (2 M) was added, followed by homogenization and centrifugation (3000 × *g* for 5 min). An amount of 100 μL of supernatant was recovered and 100 μL of 1.2% benzoic acid was added, followed by 1.4 mL of ultrapure water addition. 50 μL of the sample was mixed with 50 μL of the o-phthaldialdehyde (OPA) reagent solution for 1 min and immediately analyzed by HPLC.

Free amino acids were determined by a HPLC (Shimadzu, Kyoto, Japan) equipped with a 5- μm reversed-phase C18 columm Supelco (150 × 4.6 mm i.d. from Sigma), a Supelco guard column, Ascentis C18 (20 × 4.0 mm i.d. from Sigma) and a fluorescence detector (RF-10AXL) monitored excitation and emission wavelengths at 340 and 455 nm, respectively. All analyses were performed in gradient condition through 0.1 M sodium acetate (pH 7.2) and methanol as mobile phase.

2.4. Statistical analyses

The microbial growth curves were modeled using the DMFit 2.0 (IFR, Norwich, UK) statistical program based on predictive microbiology and idealized by Baranyi and Roberts (1994). One-way ANOVA were applied to microbial growth parameters and physico–chemical parameters from each packaging condition over the 22-day of storage as well as to identify differences in the total amount of microorganism along the microbial growth curve and the total amount of metabolites (pH, TMA-N, TVB-N, TBARS, ammonia and biogenic amines) produced over the storage time. When a significant *F* was found, additional post-hoc tests with Tukey adjustment were performed. Calculations of the integrated total amount of metabolites (pH, TMA-N, TVB-N, TBARS, ammonia and biogenic amines) produced over the storage time and total amount of microorganism along the microbial growth curve for each packaging condition [area under the curve (AUC)] was determined by the use of a trapezoidal method (baseline concentration: $y = 0$). Statistical significance was set at the 0.05 level of confidence. All analyses were performed using GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego California USA).

3. Results and discussion

3.1. Proximate composition

Average moisture, protein, lipid and ash contents of the rainbow trout flesh were 71.38 ± 0.50, 18.28 ± 1.19, 8.26 ± 0.52 and 1.16 ± 0.02%, respectively. Our results are in accordance with previous studies in rainbow trout which reported to moisture, protein, lipid and ash contents ranging from 73.38 to 76.99%, 18.7–20.83%, 1.83–5.11% and 0.91–1.35%, respectively (Asghari, Zeynali, & Sahari, 2013; Fallah, Siavash Saei-Dehkordi, & Nematollahi, 2011; Gokoglu, Yerlikaya, & Cengiz, 2004). However a higher amount of lipids was observed in the present study which may be explained by a high-lipid diet and restricted activity of farmed rainbow trout (Fallah et al., 2011; González-Fandos, Villarino-Rodrıguez, Garcıa-Linares, Garcıa-Arias, & Garcıa-Fernández, 2005). Furthermore, it is well documented that lipid composition of fish varies and depends on factors such as diet composition, fish size, age, sex and environmental conditions (Fallah et al., 2011; Huss, 1995). Several studies have shown the variation in the lipid content between farmed and wild fish ranging from 1.28 to 5.11% (Asghari et al., 2013; Fallah et al., 2011; Gokoglu et al., 2004; Jouki, Mortazavi, Yazdi, Koocheki, & Khazaei, 2014; Pezeshk, Rezaei, & Hosseini, 2011).

3.2. Microbial growth during refrigerated storage

The changes in total aerobic mesophilic count (TAMC), total aerobic psychrotrophic count (TAPC) and Enterobacteriacea count in trout fillets as a function of packaging conditions and storage

time are shown in Fig. 1A–C.

The TAMC, TAPC and Enterobacteriacea initial counts (day 0) of rainbow trout fillets were 3.80 ± 0.20 log cfu g^{-1}, 3.60 ± 0.06 log cfu g^{-1} and 2.30 ± 0.30 log cfu g^{-1}, respectively, that were considered a relatively low initial microbial load. The initial microbial count of freshwater fish depends on several factors such as species; water temperature and conditions; good agricultural and manufacturing practices; and transportation conditions, which can make the initial microbiological counts vary from 10^2-10^6 CFU/g (Arashisar et al., 2004; Chytiri, Chouliara, Savvaidis, & Kontominas, 2004; Frangos, Pyrgotou, Giatrakou, Ntzimani, & Savvaidis, 2010; Jouki, Yazdi, Mortazavi, Koocheki, & Khazaei, 2014; Mexis, Chouliara, & Kontominas, 2009;). In this study, the fresh and untreated fish presented high initial microbiological quality, considering the microbiological maximum limit (7 log cfu g^{-1}) as recommended by the International Commission on Microbiological Specifications for Foods (ICMSF, 1998). This limit was also considered for total aerobic mesophilic count as a microbiological criterion to indicate the shelf life of fillets during the storage time.

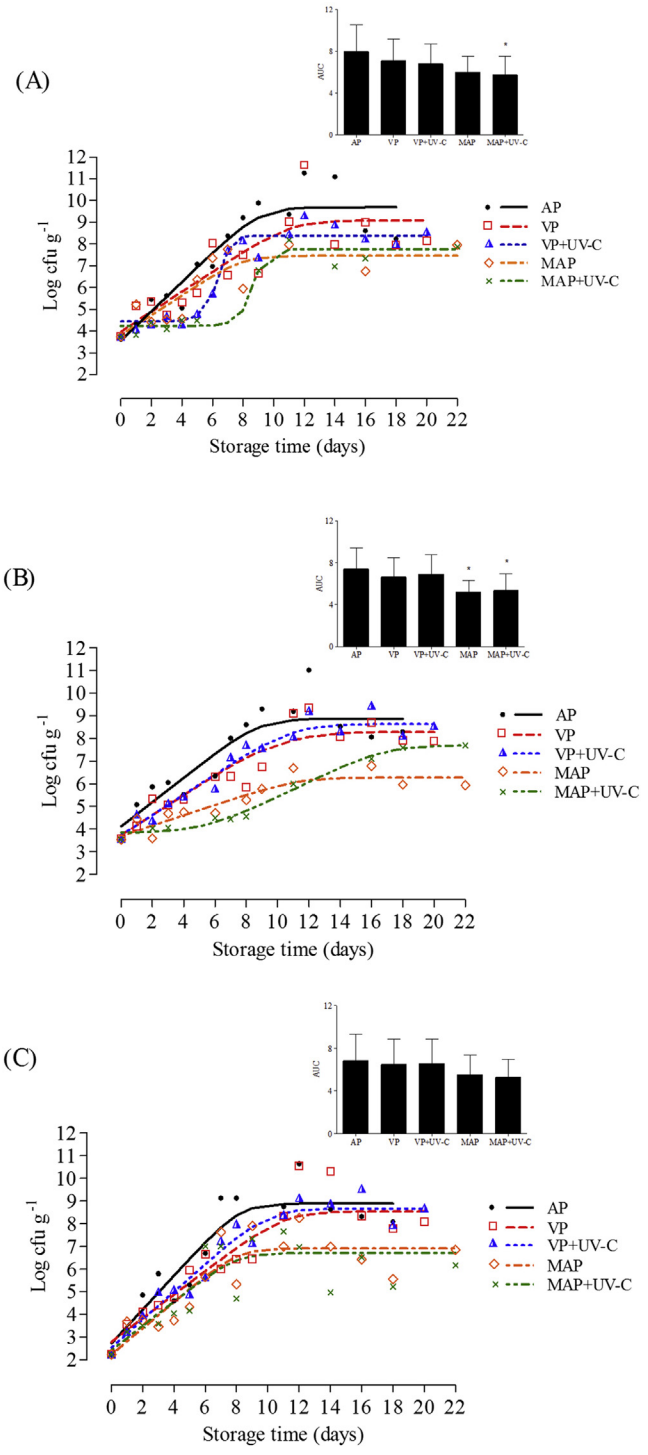

Fig. 1. Bacteriological parameters (A) Total Aerobic Mesophilic Count (TAMC); (B) Total Aerobic Psychrotrophic Count (TAPC) and (C) Enterobacteriaceae in rainbow trout fillets storage at 4 °C under different packaging conditions. AP- aerobic packaging; VP- vacuum packaging; VP + UV-C- vacuum packaging + UV-C radiation; MAP- modified atmosphere packaging; MAP + UV-C- modified atmosphere packaging + UV-C radiation. UV-C: 106.32 mJ/cm^2. MAP: 80% CO_2 and 20% N_2. AUC: * Significantly different from AP.

The analysis of mesophilic microorganism growth indicates that the packaging conditions affected shelf life differently for both groups. The values reached the upper limit of 7 log cfu g^{-1} on the 5th day of storage in the control group (AP), on the 7th day in VP and VP + UV-C and on the 11th day in MAP and MAP + UV-C. MAP affected the shelf-life positively whereas UV-C did not influence. Corroborating our results, Arashisar et al. (2004) reported that after 6 days in air, psychrotrophic and mesophilic counts reached above than 10^7 and 10^6 CFU/g respectively.

The growth parameters as a function of microbial groups and packaging conditions are shown in Table 1. When analyzing the data of growth parameters, a lag phase formation in VP + UV-C and MAP + UV-C was observed in the mesophilic group, as well as in MAP + UV-C of the psychrotrophic group, indicating that the lag phase formation can be arising from the application of UV-C radiation, since in VP and MAP conditions it was not observed. However, besides exposure to UV light action (Lazaro et al., 2014), changes in the level of CO_2 can also influence the formation and duration of lag phase (Rodriguez, Junior, Carneiro, Franco, & Mano, 2014; Simões et al., 2014) and the combined effect and mutual action of these technologies may contribute more effectively to promotion a longer adaptation time for these microbial groups, extending the lag phase and, consequently, shelf-life.

Except for the mesophilic group in VP + UV-C and MAP + UV-C, in general, the doubling times of mesophilic and psychrotrophic groups for all packaging conditions were higher than the control group (AP), suggesting that conservation technologies were effective in delaying the rate of microbial growth of these microbial groups. Furthermore, a shorter duplication time was observed for VP + UV-C and MAP + UV-C in mesophilic group, when comparing all packaging conditions. This phenomenon can be explained by the fact that the UV-C radiation acts only on the surface and does not penetrate the food in its entirety (Lazaro et al., 2014). Therefore, only the microorganisms directly exposed to UV-C source are affected and injured at DNA level (Guerrero-Beltrán & Barbosa-Cánovas, 2004). According to Guerrero-Beltrán and Barbosa-Cánovas (2004) and Sastry, Datta, and Worobo (2000), the effect of UV-C radiation is variable and depends on food surface topography and composition; the location and sensitivity of the microorganism; the strain and density of microorganisms; and even the power and wavelength of the UV-C lamp. Consequently, the slower microbial generation time may be related to the reduction of microbial competition for nutrients, which multiplies the more resistant UV-C radiation microbiota and microbiota protected from UV-C action. Furthermore, it is known that UV-C radiation causes biochemical changes in foods, such as the degradation of proteins (Koutchma et al., 2009), which potentially increases the availability of nutrients to remaining microorganisms and stimulating microbial growth (Lazaro et al., 2014). These facts lead to normalization and even enhancement of microbial growth, reducing microbial

Table 1
Growth parameters of total aerobic mesophilic count, total aerobic psychrotrophic count and enterobacteria in samples of rainbow trout fillets storage at 4 °C under different packaging conditions.

Microorganism	Parameter	AP	VP	VP + UV-C	MAP	MAP + UV-C
TA Mesophilic Count	Lag	0.00 ± 0.00	0.00 ± 0.00	5.35 ± 0.68^{b}	0.00 ± 0.00	7.73 ± 0.92^{a}
	Log	0.45 ± 0.02^{b}	0.66 ± 0.03^{a}	0.15 ± 0.07^{c}	0.70 ± 0.06^{a}	0.14 ± 0.08^{c}
	NC	9.69 ± 0.43^{a}	9.08 ± 0.27^{ab}	8.38 ± 0.03^{bc}	7.46 ± 0.15^{d}	7.77 ± 0.14^{cd}
TA Psychrotrophic Count	Lag	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	5.29 ± 0.20^{a}
	Log	0.56 ± 0.03^{c}	0.70 ± 0.12^{bc}	0.68 ± 0.07^{bc}	1.32 ± 0.05^{a}	0.91 ± 0.07^{b}
	NC	8.87 ± 0.52^{a}	8.28 ± 0.23^{ab}	8.63 ± 0.08^{a}	6.28 ± 0.02^{c}	7.69 ± 0.09^{b}
Enterobacteria	Lag	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	Log	0.40 ± 0.01^{a}	0.58 ± 0.06^{a}	0.49 ± 0.02^{a}	0.53 ± 0.08^{a}	0.44 ± 0.23^{a}
	NC	8.90 ± 0.26^{a}	8.55 ± 0.41^{a}	8.67 ± 0.23^{a}	6.92 ± 0.10^{b}	6.74 ± 0.17^{b}

TA Mesophilic Count – Total Aerobic Mesophilic Count; TA Psychrotrophic Count – Total Aerobic Psychrotrophic Count; Lag – lag phase (days); Log – log phase (hours); NC – Number of colonies in the stationary phase (log cfu g^{-1}); AP- aerobic packaging; VP- vacuum packaging; VP + UV-C- vacuum packaging + UV-C radiation; MAP- modified atmosphere packaging; MAP + UV-C- modified atmosphere packaging + UV-C radiation.
Values are displayed as mean ± SD. Different letters in the same row indicate significant differences ($P < 0.05$).

generation time and increasing microbial growth rate. Despite these facts, UV-C radiation did not affect shelf life of fillets.

Overall, the effect of carbon dioxide in reducing microbial growth rate and lowering cell count during the stationary phase of MAP and MAP + UV-C when compared to control group was evident. This result is in agreement with Simões et al. (2014) and Monteiro et al. (2013) who observed respectively that MAP conditions increased doubling time of mesophilic and psychrotrophic counts of freshwater prawns and *Oreochromis niloticus* as well as corroborate with Turan and Kocatepe (2013) and Özogul, Polat, & Özogul (2004), who also reported high concentrations of CO_2 decreasing microbial cell count of sea bass and rainbow trout respectively, increasing shelf life. This result may be explained by the bacteriostatic property of CO_2 via the modification of cell membrane function, penetration of microbial membranes, enzyme inhibition and change in physic-chemical properties of proteins (Sivertsvik et al., 2002). There was also a significant difference ($P < 0.05$) observed in the amount of total mesophilic and psychrotrophic microorganisms in MAP as well as in MAP and MAP + UV-C, as shown by AUC graph, respectively, compared to control packaging condition (AP). This result indicates that MAP applied individually (MAP) and MAP applied in combination with UV-C radiation (MAP + UV-C) was effective in reducing the total microbial count over the entire storage time.

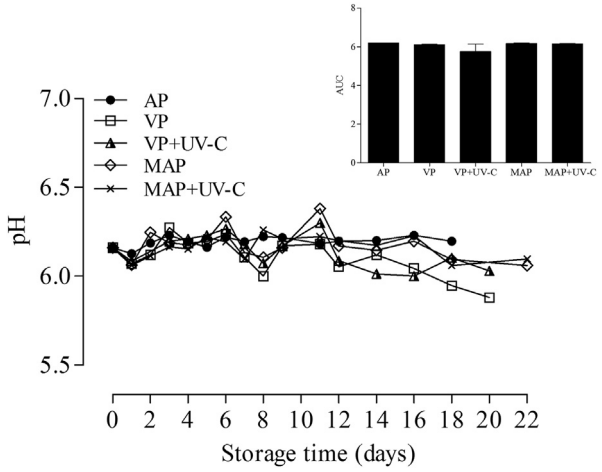

Fig. 2. Changes in pH values of rainbow trout fillets storage at 4 °C under different packaging conditions. AP- aerobic packaging; VP- vacuum packaging; VP + UV-C- vacuum packaging + UV-C radiation; MAP- modified atmosphere packaging; MAP + UV-C- modified atmosphere packaging + UV-C radiation. UV-C: 106.32 mJ/cm^2. MAP: 80% CO_2 and 20% N_2.

3.3. Physicochemical quality parameters

3.3.1. pH

Changes in pH values during storage are showed in Fig. 2. The pH initial value (day 0) of rainbow trout fillets was 6.16 ± 0.01. The post mortem pH value of fish can vary between 6.0 and 7.0, depending on factors such as species and season (Simeonidou, Govaris, & Vareltzis, 1998). Changes in pH values were observed in all packaging conditions except the control group that did not alter ($P > 0.05$) throughout the storage period. A slight decrease ($P < 0.05$) from first day in pH average was observed in all samples except AP. This fact is related to CO_2 solubility and absorption in muscle where it is converted in carbonic acid leading to acidification and decrease of pH (Banks, Nickelson, & Finne, 1980). This phenomenon was also observed by Giménez, Roncalés, and Beltrán (2002) as well as by Stamatis and Arkoudelos (2007).

3.3.2. Trimethylamine (TMA-N) and total volatile bases (TVB-N)

The TMA-N and TVB-N values for the packaging conditions during storage are presented in Fig. 3A and B, respectively. The initial values of TMA-N and TVB-N (day 0) was 0.38 ± 0.10 mg TMA-N/100 g and 5.44 ± 0.21 mg of TVB-N/100 g of tissue, which represents a good quality of rainbow trout, corroborating with relatively low initial TAMC (3.80 ± 0.20 log cfu g^{-1}). Initial TMA-N and TVB-N values in the present study are lower than those reported in rainbow trout fillets by Jouki, Yazdi, et al. (2014) (1.12 mg TMA-N/100 g and 8.23 mg of TVB-N/100 g) and Frangos et al. (2010) (1.3 mg TMA-N/100 g and 20.5 mg of TVB-N/100 g) as well as in whole gutted rainbow trout by Pezeshk et al. (2011) (12.6 mg of TVB-N/100 g). A TMA-N and TVB-N limit value of 5 mg TMA-N/100 g and 25 mg TVB-N/100 g was proposed by Masniyom, Benjakul, and Visessanguan (2002) and Giménez et al. (2002) as the beginning of spoilage for filleted rainbow trout and sea bass, respectively. Throughout the entire storage period, no packaging conditions exceeded the acceptable limits: the maximum value achieved of 4.12 ± 1.22 mg TMA-N/100 g at 22nd day and 22.30 ± 0.43 mg TVB-N/100 g at 18th day of storage in control group.

A long initial stability phase in TMA-N values for all packaging conditions was observed (Fig. 3A). Our results are in accordance to those observed by Chytiri et al. (2004) who recorded an extended period of stability in whole and fillet rainbow trout samples of 15 and 9 days with subsequent increase TMA values reaching 6.38 ± 0.05 mg TMA-N/100 g and 4.29 ± 0.13 mg TMA-N/100 g, respectively, on the 18th day of storage. This response reflects the low level of trimethylamine oxide (TMAO) in flesh of this

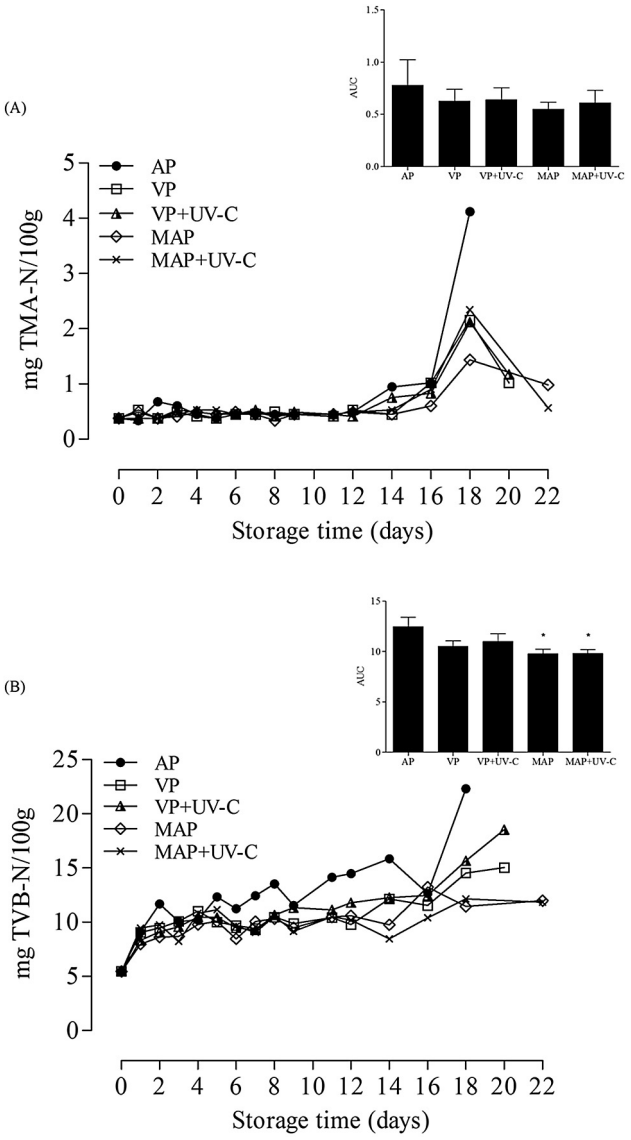

Fig. 3. Changes in (A) TMA-N and (B) TVB-N values of rainbow trout fillets storage at 4 °C under different packaging conditions. AP- aerobic packaging; VP- vacuum packaging; VP + UV-C- vacuum packaging + UV-C radiation; MAP- modified atmosphere packaging; MAP + UV-C- modified atmosphere packaging + UV-C radiation. UV-C: 106.32 mJ/cm^2. MAP: 80% CO_2 and 20% N_2. AUC: * Significantly different from AP.

freshwater fish species, leading to low TMA-N production (Chytiri et al., 2004).

An increase from the first day ($P < 0.05$) in TVB-N values was observed for all packaging conditions, although samples subject to MAP and MAP + UV-C deteriorated more slowly when compared to other groups (Fig. 3B). Giménez et al. (2002) observed the same phenomenon in samples submitted to MAP. According TVB-N results, TMA-N values from samples subject to MAP and MAP + UV-C also increased more slowly, only starting to increase ($P < 0.05$) after 18 and 16 days of storage, respectively.

According to Masniyom (2011) and Banks et al. (1980), higher CO_2 concentration potentially inhibited the growth of mainly gram negative microorganisms and decreased deamination capacity of bacteria, resulting in lower volatile compounds production. This fact corroborates our microbiological results (Table 1), since samples submitted to MAP and MAP + UV-C present in general a higher doubling time and lower number of colonies in the stationary phase. In addition, there was a significant difference ($P < 0.05$) in the amount of total TVB-N (AUC) produced in MAP and MAP + UV-C samples, which reasserts the efficacy of these technologies to reduce TVB-N production during storage and to extend shelf life.

3.3.3. Lipid oxidation – thiobarbituric acid reactive substances

The TBARS values during storage are shows in Fig. 4. The initial values of TBARS (day 0) was found to be 0.07 ± 0.01 mg MA/Kg of tissue which was similar to the value reported for rainbow trout by Rezaei, Hosseini, Langrudi, Safari, and Hosseini (2008) (nearly 0.04 mg MA/Kg) as well as lower than the values reported by Jouki, Yazdi, et al. (2014) (0.12 mg MA/Kg) in rainbow trout fillets and by Pezeshk et al. (2011) (0.33 mg MA/Kg) in whole gutted rainbow trout. The TBARS value of groups submitted to MAP and MAP + UV-C began to increase ($P < 0.05$) from the 9th day of storage, jointly with the control group, while samples subjected to VP and VP + UV-C increased ($P < 0.05$) only at the 16th of storage. Similar results have been reported in rainbow trout (Giménez et al., 2002), chub mackerel (Erkan, Özden, & Inuğur, 2007) and sea bass (Turan & Kocatepe, 2013) which also verified higher TBARS values in samples submitted to MAP conditions as well as lower TBARS values in vacuum-packed samples. These results can be explained due to CO_2 performance that causes denaturing of muscle proteins leading to liberation of iron that acts as pro oxidant in the lipid fraction causing the decrease of shelf life of samples submitted to MAP (Masniyom, 2011). Moreover, the presence of oxygen has a definite influence on the level of lipid oxidation (Giménez et al., 2002) and the absence of this compound, such as vacuum treated packages (VP and VP + UV-C), results in lower TBARS values. In addition light irradiation, one of the pre-determining factors for enhancement of lipid oxidation (Namiki, 1990), caused no significant changes ($P > 0.05$) in TBARS values of the present study, potentially because of the low UV-C dose and exposure period which was not able to promote oxidation. Due to lack of information about the effects of UV-C radiation in fish, our results were compared to previous report in chicken meat (Lazaro et al., 2014) who also observed that UV-C radiation applied by the similar exposure time did not affect the TBARS values. A fluctuation behavior of TBARS values was observed during storage time and it is related to the non-stability of malondialdehyde compound that could bond to others constituents of fish such as nucleic acids,

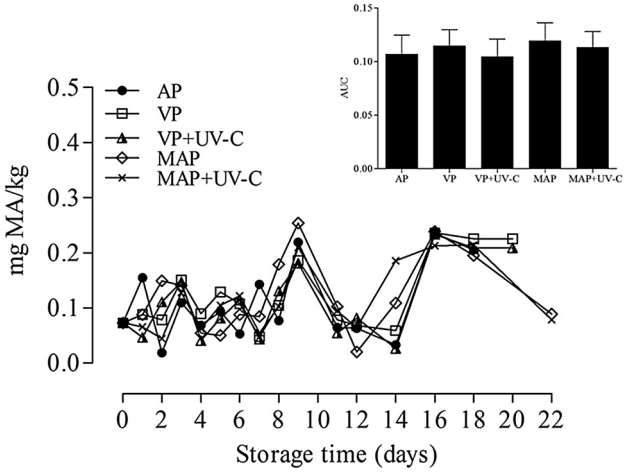

Fig. 4. Changes in TBARS values of rainbow trout fillets storage at 4 °C under different packaging conditions. AP- aerobic packaging; VP- vacuum packaging; VP + UV-C- vacuum packaging + UV-C radiation; MAP- modified atmosphere packaging; MAP + UV-C- modified atmosphere packaging + UV-C radiation. UV-C: 106.32 mJ/cm^2. MAP: 80% CO_2 and 20% N_2.

protein, amino acids (Fernandez, Perez-Alvarez, & Fernandez-Lopez, 1997) resulting in a decrease in TBARS values.

A maximum level of TBARS value of 5 mg MA/Kg of tissue was proposed to indicate good quality for Pacific salmon (*Oncorhynchus nerka*) by Ibrahim Sallam (2007). Our results were found to be much lower than the proposed limited throughout 22 days of storage in all packaging conditions, a result that is in accordance with others studies in rainbow trout fillets (Jouki, Yazdi, et al., 2014; Pezeshk et al., 2011).

3.3.4. Ammonia

The ammonia values during storage are shows in Fig. 5. The initial values of ammonia (day 0) were found to be 5.26 ± 0.08 μg NH_3/g of tissue.

A significant decrease ($P < 0.05$) was observed at the beginning of the storage period from 4th day with subsequent increase ($P < 0.05$) at the end of the storage period from 16th and 18th days for AP and VP + UV-C, respectively. A significant decrease ($P < 0.05$) was observed at the beginning of the storage period from the 5th day for VP and MAP samples and from the 4th day of storage for MAP + UV-C samples, remaining constant until the end of storage. Malondialdehyde is known to bond to other constituents of fish, such as the amino acids degradation product that are ammonium compounds (Fernandez et al., 1997). This process could explain the decrease in values of all samples mainly at the beginning of the storage. At the end of storage, AP and VP + UV-C had a higher concentration, startling malondialdehyde-ammonia bonds, which resulted in higher values. The ammonia values corroborate with TVB-N values which reached the highest production at the end of storage period, mainly in AP and VP + UV-C samples. The action of UV-C radiation facilitates enzymes and microbial action causing that samples submitted to VP + UV-C had a similar result of the control group.

The same initial decrease phenomenon was found in MAP and MAP + UV-C samples; however, MAP mode of action promotes extension shelf life maintaining low concentration of ammonia during storage time. The lower ammonia production corroborates our microbiological results (Table 1), since samples submitted to MAP and MAP + UV-C in general present a higher doubling time and lower number of colonies in the stationary phase. This behavior was confirmed also by AUC graph (Fig. 5) since a significant difference ($P < 0.05$) was observed for the total ammonia production in MAP samples when compared to control group (AP), indicating that MAP was effective in reducing ammonia production during storage.

3.3.5. Biogenic amines

The values of putrescine and cadaverine values during storage are shows in Fig. 6A and B, respectively. The initial values (day 0) of putrescine (Fig. 6A) were found to be 0.88 ± 0.02 mg/kg of tissue which corroborates to previous studies in rainbow trout stored on ice (0.42 ± 0.17 mg/kg) (Rezaei et al., 2007) and in non-vacuum-packed carp (0.70 mg/kg) (Kří;žek et al., 2004). A significant increase ($P < 0.05$) was observed in samples submitted to MAP, MAP + UV-C and VP from the 3rd day of storage, while control group (AP) and VP + UV-C samples began to increase ($P < 0.05$) from the 2nd day of storage. A large and rapid increase of

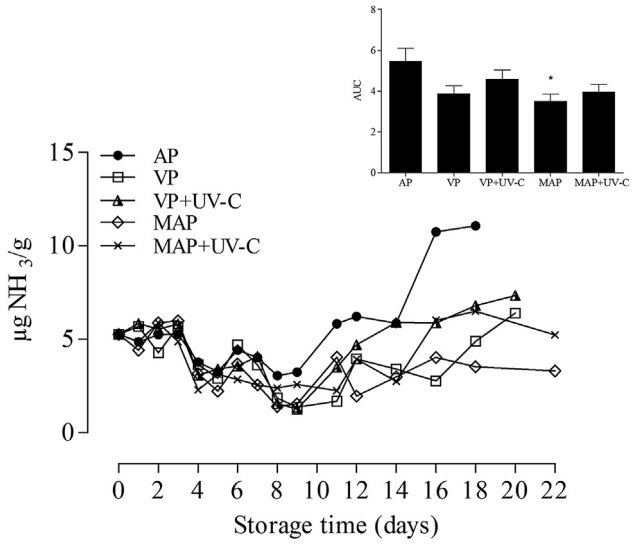

Fig. 5. Changes in ammonia values of rainbow trout fillets storage at 4 °C under different packaging conditions. AP- aerobic packaging; VP- vacuum packaging; VP + UV-C- vacuum packaging + UV-C radiation; MAP- modified atmosphere packaging; MAP + UV-C- modified atmosphere packaging + UV-C radiation. UV-C: 106.32 mJ/cm^2. MAP: 80% CO_2 and 20% N_2. AUC: * Significantly different from AP.

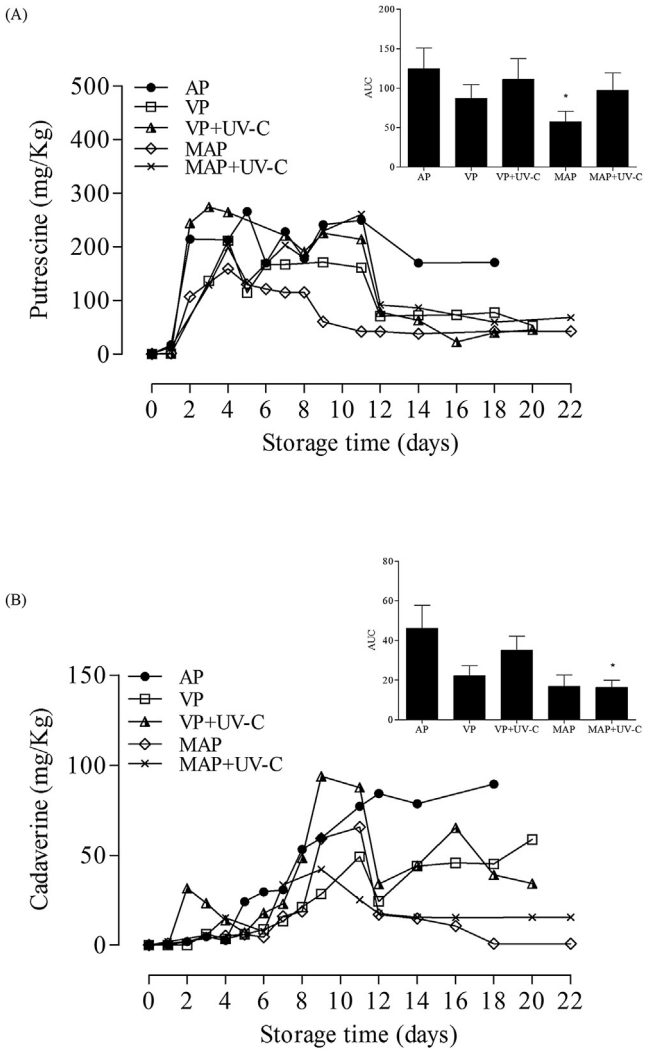

Fig. 6. Changes in (A) Putrescine and (B) Cadaverine values of rainbow trout fillets storage at 4 °C under different packaging conditions. AP- aerobic packaging; VP- vacuum packaging; VP + UV-C- vacuum packaging + UV-C radiation; MAP- modified atmosphere packaging; MAP + UV-C- modified atmosphere packaging + UV-C radiation. UV-C: 106.32 mJ/cm^2. MAP: 80% CO_2 and 20% N_2. AUC: * Significantly different from AP.

putrescine production was observed in the initial period of storage for all packaging conditions reaching high values with subsequent reduction during storage time. This rapid increase production is in accordance to others studies (Katikou, Georgantelis, Paleologos, Ambrosiadis, & Kontominas, 2006; Rezaei et al., 2007) and to findings of our microbiological analyses, indicating that it may be related to a large use of amino acid precursor (ornithine and arginine) of putrescine (Chytiri, Paleologos, Savvaidis, & Kontominas, 2004; Silla Santos, 1996) by psychotropic microorganisms, specifically *Pseudomonas* spp. (Gram & Dalgaard, 2002), in the initial of the microbial growth curve. In addition a large amount of free precursor amino acids of putrescine arginine and ornithine at day 0 was observed in rainbow trout fillets, with values of 237.88 ± 26.11 mg/kg and 22.26 ± 10.51 mg/kg, respectively. According to Silla Santos (1996), amino acid arginine can be metabolized to ornithine, which is posteriorly converted by bacteria decarboxylation in putrescine explaining the large production of this amine.

An earlier and intense putrescine production was observed in samples submitted to VP + UV-C, attaining high values in the initial days of storage. The increased availability of free amino acids by radiation leads to faster biogenic amines production to decarboxylase microorganisms (Gram & Dalgaard, 2002; Koutchma et al.,2009). This fact can be confirmed through the lower doubling time and larger number of colonies in the stationary phase of psychotropic microorganisms in samples subjected to VP + UV-C, suggesting faster microbial growth and amines production (Lazaro et al., 2014).

A late and lower putrescine production was respectively observed in samples submitted to VP and MAP (Fig. 6A). It is common knowledge that CO_2 presence and shortage of oxygen inside the package inhibits microbial growth, which reduces spoilage and increases the shelf life of fish (Özogul, Polat, & Özogul, 2004; Simões et al., 2014; Sivertsvik et al., 2002). This fact can be verified in Table 1, overall, by all microbial groups. Samples submitted mainly to MAP showed higher doubling time and lower number of colonies in the stationary phase, especially psychrotrophic microorganisms. Furthermore, the total amount of putrescine production during all storage time (AUC) was significantly lower ($P < 0.05$) in samples submitted to MAP, indicating that MAP is effective in reducing putrescine production.

Cadaverine was not detected and free precursor amino acids of cadaverine lysine achieved 32.48 ± 3.18 mg/kg in day 0. A significant increase ($P < 0.05$) in values was observed on the 2nd and 3rd days to samples submitted to VP + UV-C and MAP + UV-C; and on the 4th and 6th days for samples submitted to MAP and VP, respectively. Our results corroborates to previous studies in fish that did not detect cadaverine in the initial days of storage and observed a late elevation of values (Emborg, Laursen, Rathjen, & Dalgaard, 2002; Paleologos, Savvaidis, & Kontominas, 2004; Rodrigues et al., 2013). Similar results to putrescine behavior was observed in cadaverine parameter in the samples subjected to UV-C radiation (VP + UV-C), which presented an earlier production and greater amount of cadaverine during all storage time (AUC), as well as in samples subjected to VP, MAP and MAP + UV-C that demonstrated a later production and lower amount of cadaverine during all storage time (AUC).

However, cadaverine formation has a strong relationship with Enterobacteriaceae spp. count (Katikou et al., 2006) despite also having relationship with *Pseudomonas* spp. (Paleologos et al., 2004; Rezaei et al., 2007). Microbiological analyzes results (Table 1) are corroborating with the results of cadaverine parameter. In general, samples submitted mainly to MAP and MAP + UV-C showed lower number of colonies in the stationary phase, especially Enterobacteria. Furthermore, the total amount of cadaverine production during all storage time (AUC) was significantly lower ($P < 0.05$) in samples submitted to MAP + UV-C radiation, indicating that the combination of these two technologies is effective in reducing cadaverine production. To the best of our knowledge, there is a lack of information regarding the evaluation of the behavior of biogenic amines in fish treated by UV-C radiation. Therefore, our results were compared to previous report in chicken meat (Lazaro et al., 2014) which also observed that UV-C radiation (175.5 mJ/cm^2) increased biogenic amines production during refrigerated storage.

4. Conclusion

This study demonstrated that MAP (80% CO_2/20% N_2) enhanced the shelf-life of rainbow trout fillets at least twice by retarding microbial growth parameters and delaying chemical changes. Beside UV-C radiation (106.32 mJ/cm^2) did not affect shelf life, it promoted lag phase in mesophilic and psychrotrophic groups. Regarding physical–chemical parameters, MAP reduced total production of ammonia, TVB-N and putrescine, whereas MAP + UV-C decreased TVB-N and cadaverine production during entire storage period.

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