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Incorporation of pink pepper residue extract into chitosan film combined with a modified atmosphere packaging: Effects on the shelf life of salmon fillets

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ABSTRACT

Fresh salmon safety and quality is a major concern of consumers. In the current research, the effects of chitosan films incorporated with pink pepper residue extract and combined with modified atmosphere packaging (100% CO_2) on quality properties of skinless salmon fillets during refrigerated storage (2 °C) were evaluated in the course of 28 days. Two different treatments as chitosan film (CF) and chitosan film incorporated with pink pepper residue extract (CFPP) and a control were compared. Salmon fillets were assessed for physicochemical (pH, WHC, TPA, Cie L*a*b*, TMA, TBA, value K), microbiological (mesophilic and psychrotrophic count, and lactic acid bacteria) and sensory properties. The results showed that CF and CFPP significantly reduced lipid oxidation relative to the control. Bacterial counts were significantly lower in CFPP, contributing to the significant reduction of trimethylamine. For sensory evaluation, CF and CFPP presented satisfactory results of off-odor and overall appearance. Despite being similar to the control, CFPP showed the lowest off-odor score. The results indicated that CFPP were more effective in maintaining the quality of salmon fillets during refrigerated storage.

1. Introduction

World trade of salmon has increased in the last decades and the demand for farmed Atlantic salmon has grown considerably. The consumption of fish has also increased because of its nutritional characteristics as well as its benefits to the consumer health (FAO, 2016).

Considering these facts, several fish products have been processed by new markets, such as sushi, sashimi, and carpaccio (Gómez-Estaca et al., 2018). All these processed products are mainly manufactured by using fresh fish, but usually the fish is sold frozen in the market, having a considerable influence on freshness and commercial value (Wu, Yang, Zhou, Lai, & Zhong, 2019).

However, fresh salmon is a highly perishable product and has a very short storage life, which limits the distribution and marketing of fresh salmon (Gómez-Estaca et al., 2018). Spoilage of fresh fish during storage is mainly caused by enzymatic reactions (microbial and autolytic degradation), thus generating off-flavors (Powell, Ratkowsky, & Tamplin, 2015). Additionally, lipid oxidation of polyunsaturated fatty acids is one of the limiting factors of shelf life. The oxidation produces volatiles compounds that affect sensory properties (Jacobsen, 2019), influencing the acceptance of fresh salmon.

Therefore, conservation techniques are the main allies of fish companies because they maintain food safety and the sensory appeal of the fresh salmon (Soares, Silva, Barbosa, Pinheiro, & Vicente, 2017). The packaging system of "fish products" has improved, especially with the use of modified atmosphere packaging (MAP) (Bouletis, Bouletis, Arvanitoyannis, & Hadjichristodoulou, 2017). When fresh salmon is stored under a modified atmosphere without oxygen, consequently the lipid oxidation and growth of aerobic bacteria can be significantly decreased (Hansen et al., 2009; Macé et al., 2012).

The effect of MAP on the storage life of fresh fish is conditioned by the material packaging, pressure, concentration of CO_2 available in the

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package and storage temperature (Rotabakk & Sivertsvik, 2012). High CO_2 levels decreases the number of aerobic bacteria, however this levels of CO_2 favor the selection of lactic acid bacteria (LAB) (Françoise, 2010). LAB, particularly *Lactococcus piscium* and *Hafnia alvei,* are some of the spoilage bacteria of salmon stored under modified atmosphere (Macé et al., 2012).

Other conservation techniques have been extensively studied. Some authors have investigated the application of natural antioxidants on fish preservation (Albertos et al., 2017; Jouki, Yazdi, Mortazavi, Koocheki, & Khazaei, 2014). However, the addition of natural compounds may affect the sensory characteristics of the product, such as palatability (Kostaki, Giatrakou, Savvaidis, & Kontominas, 2009). Thus, the addition of natural compounds in biopolymeric films may be a good alternative to increase sensory acceptance and enable the gradual release of these compounds on the food surface during storage (Campos, Gerchenson, & Flores, 2011).

Chitosan films have been incorporated with active and bioactive compounds to enhance the antimicrobial and antioxidant effects. Thus, these (bio)active films can increase the shelf life or even improve the sensorial properties of the food (Ganiari, Choulitoudi, & Oreopoulou, 2017) in order to improve or maintain the intrinsic quality of meat, poultry and seafood (Umaraw & Verma, 2017). Chitosan is a cationic polysaccharide obtained from the deacetylation of chitin. It has been used as environmentally friendly packaging material as it is biodegradable, biocompatible and non-toxic. In addition, chitosan was shown to have antimicrobial activities (Fan et al., 2009; Souza et al., 2010).

The food processing industry generates large amounts of waste and many of these residues present antimicrobial and antioxidant activity (Makris, Boskou, & Andrikopoulos, 2007) such as pink pepper residue (*Schinus terenbithifolius* Raddi). *S. terebinthifolius* is a plant native to Brazil, belonging to the family Anacardiaceae, popularly known as pink pepper, aroeira, among others (Lorenzi & Matos, 2008). It is characterized by its high levels of bioactive compounds, such as ascorbic acid, phenolic compounds, flavonoids and carotenoids (Alvarez-Parrilla, La Rosa, Amarowicz, & Shahidi, 2011). The addition of pink pepper residue to the chitosan films demonstrated antioxidant activity, and consequently, reduced the lipid oxidation of chicken burger (Serrano-León et al., 2018).

The purpose of this study was to determine the efficacy of modified atmosphere packaging containing 100% CO_2 and chitosan films incorporated with pink pepper residue extract on the quality characteristics and the shelf life of Atlantic salmon fillets.

2. Material and methods

2.1. Preparation of pink pepper residue extract

Agro-industrial residues of pink pepper (mixture of peel, skin, stems, and pulp) were dried in an oven with forced air circulation (Nova Ética 400/D, Vargem Grande Paulista, Brazil) at 40 °C for 24 h, subsequently ground (Quimis Q298A21, Diadema, São Paulo, Brazil) to produce the size of < 0.5 mm. One gram of dried and ground residue was macerated with 10 ml of a 80% (*v*/v) ethyl alcohol solution, on a rotary shaker (Nova Ética 304D, Vargem Grande Paulista, São Paulo, Brazil), under constant mechanical agitation at room temperature and protected from light for 48 h. The extract was filtered (12.5 mm qualitative filter paper), and the filtrate obtained was concentrated in a vacuum rotary evaporator (Tecnal TE-210, Piracicaba, São Paulo, Brazil) at 65 °C. The residue was dissolved in distilled water (50 ml) and the extract was stored in amber glass bottles and kept at −80 °C until the manufacture of the films (Bergamaschi, 2016).

2.2. Measurement of total phenolic content

Total phenolic content (PC) of pink pepper residue was estimated using the Folin-Ciocalteu's phenol reagent, in triplicate, following the spectrophotometric method proposed by Al-Duais, Müller, Böhm, and Jetschke (2009). The extract solutions (0.1 ml) were mixed with 5 ml of distilled water and 0.5 ml of Folin-Ciocalteau reagent. After 3 min, 1.5 ml of Na_2CO_3 (20%) and 2.9 ml of distilled water were added. The absorbance was measured in a spectrophotometer (Molecular Devices, LLC, Sunnyvale, CA, EUA) at 740 nm after 2 h of incubation. PC was expressed in gallic acid equivalent (GAE) per g (dry weight) of pink pepper residue.

2.3. Preparation of active films

The chitosan films were prepared according to (Yoshida, Oliveira & Franco, 2009). The 2% (m/v) chitosan suspension was prepared by dispersing chitosan (degree of acetylation (DA) ≥ 95%, ChitoClear FG 95 – GRAS, Primex, Iceland) in a 1% (*v*/v) glacial acetic acid solution. The suspension was homogenized by magnetic stirring (IKA, RHB1, Wilmington, USA) at room temperature for 60 min until complete dissolution. Then, glycerol (0.25% m/v) was added as plasticizer, under stirring, for 10 min. The chitosan suspension was poured (50 ml) into plastic Petri dishes (13 cm × 13 cm) and dried at 40 °C in an air-forced oven (Tecnal, TE-394/2, Brazil) for about 24 h. The pink pepper residue extract was added in the filmogenic suspension at 100 mg GAE/kg of meat (at level 0.6% v/v chitosan suspension) after the addition of glycerol solution using an Ultra-Turrax (Ika-Werke, model T25, Germany) at 20,000 rpm for 5 min. The concentration of pink pepper extract was based on a preliminary study (Serrano-León et al., 2018).

2.4. Raw materials, processing and packaging

Farmed Atlantic salmon ($n = 18$; body weight ca. 5.0 kg) was slaughtered, bleeded and gutted by Pesquera del Mar Antartico S.A (Puerto Montt, Chile). The fish was transported by refrigerated truck and delivered to the Quality and Processing of Meat Laboratory at ESALQ-USP (Piracicaba, Brazil) in polystyrene boxes under ice. The 36 salmon fillets (right and left sides; 11 days post mortem) were cut into 300 g samples and the samples divided into three treatments as follows: skinless salmon with absorbent pad placed at the upper of the trays (CT; control); skinless salmon with chitosan film placed at the upper of the trays (CF) and skinless salmon with chitosan film incorporated with pink pepper residue extract placed at the upper of the trays (CFPP).

The salmon fillets were placed on rigid polypropylene trays (model 13D65; size, 24 × 16 × 65 cm, oxygen permeability rate [OTR] < 0.5 cm^3/m^2/24 h at 50% relative humidity [RH], Sealed Air) and ethylene-vinyl alcohol copolymer (EVOH) was used as a barrier for O_2 and CO_2. Subsequently, the trays were gas flushed in a vacuum-packing machine (model T200, Multivac, Curitiba, Brazil). This machine creates a vacuum, then lets the gas (100% CO_2) enter, and finally seals the tray with the barrier film (model 4532-G, with a nominal thickness of 70 μm, OTR < 0.5 cm^3/m^2/24 h at 23 °C, 66% RH and water vapor permeability < 0.5 g/m^2/24 h at 38 °C and 90% RH, Bemis Company, Dixie Toga). The vacuum-packing machine was programmed to operate under the following conditions: evacuation pressure of 10 mbar; atmospheric injection pressure of 750 mbar and sealing at 168 °C for 5 s.

All samples were stored in the dark in a chilled room (2 ± 1 °C). Triplicate samples were analyzed for sensory, chemical, physical, and microbiological properties after 7, 14, 21 and 28 days of refrigerated storage. Evaluation days were selected to represent several theoretical cases of retail purchase. The best case was represented by 7 d, in which salmon fillet could be packaged at central distribution and sent to retailer and purchase after minimal illuminated display time. The worst-case was represented by 28 d after dark storage, and average purchase points were represented by 14 and 21 days.

2.5. Headspace composition

The gas composition (% v/v, CO_2/O_2) was monitored by headspace oxygen/carbon dioxide analyzer (CheckPoint II, PBI Dansensor A/S, Denmark). Gas measurements were performed by inserting the needle through a rubber septum attached on the top of the tray.

2.6. Microbiological analysis

Aerobic mesophilic and psychrotrophic bacterial viable counts were determined by diluting 25 g of sample in 225 ml of 0.1% sterile peptone water (PW) (Difco Laboratories, Detroit, MI, USA). The total count of mesophilic bacteria was performed according to a standard plate counting method - Plate Count Agar medium - PCA (KasviTM) (incubation at 35 ± 1 °C, 48 h) (Morton, 2001). The count of psychrotrophic bacteria was performed with surface plating on Plate Count Agar medium (Difco PCATM) (incubation at 7 ± 1 °C, 10 days) (Cousin, Jay, & Vasavada, 2001). Lactic acid bacteria (LAB) counts were enumerated on a MRS Agar (de Man, Rogosa and Sharpe, HiMedia code 198 M641) supplemented with potassium sorbate. Samples (0.5 ml) of appropriate serial dilutions were spread on the pre-set sterile MRS agar plates followed by overlaying with the same medium and incubated at 30 ± 1 °C for 48 h (Hall, Ledenbach, & Flowers, 2001). The determinations were performed in duplicate and the counts expressed as colony forming unit log CFU/g sample. Total and thermotolerant coliforms were analyzed using the multiple-tube fermentation test and expressed as most probable number (MPN)/g sample (Kornacki & Johnson, 2001).

2.7. pH

The pH of the salmon samples was determined from three randomly selected localizations in individual fillets using a calibrated potentiometer (pH 1140 model, Mettler-Toledo, Switzerland) with automatic temperature compensation and a glass penetration electrode (Digimed, Presidente Prudente, São Paulo, Brazil).

2.8. Water holding capacity (WHC)

The water holding capacity (WHC) was determined in triplicate by the centrifugation method described by Eide, Børresen, and Strøm (1982). The average was expressed as the percentage of water retained in the fish after centrifugation (wet weight basis).

2.9. Texture profiling analysis (TPA)

Salmon samples were subjected to texture profile analysis (TPA) using the Texture Analyzer (TA.XT2i Stable Micro Systems Ltd., Surrey, England) using the parameters described by (Einen and Thomassen, 1998). The samples were cut into cubes (2 × 2 × 2 cm). A double compression cycle test was performed up to 50% compression of the original sample height using the probe P/75 (75 mm diameter, TA.XT2i Stable Micro Systems Ltd., Surrey, England). A time of 5 s was allowed to elapse between the two compression cycles. Force–time deformation curves were obtained with a 25 kg load cell applied at a cross-head speed of 1 mm/s. Hardness (kgf), springiness (%) and cohesiveness were determined as described by Bourne (1978).

2.10. Instrumental color

The color on the surface of fillets was measured using a colorimeter Konica Minolta, Chroma Meter (8 mm-diameter aperture, Illuminant D65, 10° standard observer, CR-400, Mahwah, New Jersey, USA). Three scans from each fillet were obtained and averaged for statistical analysis. CIE L* (lightness), a* (redness) and b* (yellowness) were used to characterize the color. The equipment was calibrated using a standard white porcelain (Y = 93.7, x = 0.3160 and y = 0.3323).

2.11. Trimethylamine

Trimethylamine (TMA) was determined by the distillation method according to Brazil (1981). Ground salmon (100 g) was weighed and transferred to the blender with 300 ml of 5% trichloroacetic acid solution. The homogeneous mass was filtered on qualitative filter paper. The filtrate (5 ml) was transferred to the semi-micro Kjeldahl distillation apparatus and 5 ml of 2 mol/l sodium hydroxide solution was added and distilled for 5 min. Then, the distillate was collected in a 125 ml Erlenmeyer flask containing 5 ml of a 0.01 M hydrochloric acid with 5 drops of the 1% rosolic acid solution. A formaldehyde solution at 16% (*v*/v, 1 ml) was added to each 10 ml of liquid in the Erlenmeyer flask and titrated with a 0.01 mol/l sodium hydroxide solution until the pale pink coloration. TMA data were expressed as mg TMA per 100 g fish muscle.

2.12. Oxidative stability

The oxidative stability of the samples was determined using the 2-thiobarbituric acid method (TBA) following the methodology described by Vyncke (1970, 1975) and Sorensen and Jorgensen (1996). The standard curve was prepared with 1,1,3,3-tetraethoxypropane (TEP) with concentrations ranging from 0 to 0.4 μmol/ml of TEP. The absorbance was measured in a spectrophotometer (Shimaadzu, UV–Vis mini 1240) at 532 and 600 and the TBA content was expressed as mg of malondialdehyde/kg of sample.

2.13. Value K (%)

The concentrations of various nucleotides and their breakdown products in salmon fillets were determined using high-pressure liquid chromatography as previously described (Burns, Kee, & Irvine, 1985). The ratio of the quantity of hypoxanthine (Hx) and inosine (HxR) to the total quantity of adenosine triphosphate (ATP) and related substances (ADP – adenosine diphosphate; AMP - adenosine monophosphate; IMP - inosine monophosphate) is defined as value K (Saito, Arai, & Matsuyoshi, 1959). This ratio is used as an index and it gives a relative freshness rating of fish muscle.The conditions used for the analysis were: UV–Vis detector, C_{18} column (250 × 4.6 mm), injection of 20 μL, isocratic method, wavelength of 254 nm, mobile phase consisted of a buffered KH_2PO_4 solution at 0.06 mol/L (pH = 7.0) and a flow rate of 1.4 ml/min was used. The value K was calculated using the Eq. (1):

$$Value\ K\ (\%) = \frac{Hx + HxR}{ATP + ADP + AMP + IMP + Hx + HxR} * 100 \quad (1)$$

2.14. Fatty acid profile

Total lipids were extracted in triplicate according to the method of Folch, Lees, and Sloane-Stanley (1957). An aliquot of lipids (50 mg) were methylated according to the method proposed by Hartman and Lago (1973). Fatty acid methyl esters (FAME) were analyzed using gas chromatography (GC) (Focus CG- Finnigan) equipped with a capillary column (100 m × 0.25 mm i.d., 0.20 μm film thickness) (Varian) coupled with a flame ionization detector. GC-oven program temperature was as follows: initial temperature of 70 °C for 4 min, then it was raised to 175 °C at a rate of 13 °C/min and kept for 27 min, and finally the temperature was raised to 215 °C at a rate of 7 °C/min and kept at 230 °C for 5 min. The injector and detector temperatures were 250 °C and 300 °C, respectively. Hydrogen was used as carrier gas at a flow rate of 1.8 ml/min. Samples (1 μL) were injected using an automatic injector. Fatty acids were identified by comparing the retention times of the methyl esters of the samples with fatty acid standards (Supelco TM Component FAME Mix, cat 18,919 Supelco, EUA). The fatty acids were quantified by normalizing the areas of the methyl esters, expressed as percentage area (%).

2.15. Sensory analysis

The sensory analysis of salmon fillets (surface) was analyzed by a sensory panel composed of eight trained assessors with previous experience in sensory descriptive analysis of meat products. The panelists had previous experience in other sensory analyses performed by our group at the Quality and Processing of Meat Laboratory (Saldaña et al., 2018; Selani et al., 2016). The performance of the assessors during the training and test steps was validated by Saldaña et al. (2018). A training session (30 min) was used to prepare the sensory panel for sensory evaluation of salmon samples. The sensory evaluation was relatively simple since the panel had extensive experience in the evaluation of products of animal origin and because they evaluated only two sensory attributes (off-odor and overall appearance) of 3 samples. The experimental design was composed of 3 samples, 2 replicates, totaling 12 evaluations. The salmon samples, coded with three random numbers, were served monadically following a balanced block design to the assessors. Assessors rated the off-odor and overall appearance of each salmon fillet using a 9 cm non-structured linear scale, anchored at the ends with "minimal" on the left, and "maximum" on the right. The off-odor was evaluated by the assessors panel between 0.5 and 1 min after opening the trays.

2.16. Statistical analysis

This study was conducted in a completely randomized design with factorial arrangement (3 treatments × 4 storage times). The 3 treatments were skinless salmon (CT; control), skinless salmon with chitosan film (CF), and skinless salmon with chitosan film incorporated with pink pepper residue extract (CFPP), analyzed on 4 different storage times (7, 14, 21 and 28 days). The experimental unit was the salmon fillet packed using modified atmosphere, consisting of three independent replicates within each storage time range. Data were analyzed using ANOVA model shown in Eq. (2), considering that errors are normally distributed with mean zero and constant variance (Granato, Calado, & Jarvis, 2014; Weisberg, 2014).

$$Y_{ijk} = \mu + \tau_i + \alpha_j + \tau_i * \alpha_j + \varepsilon_{ijk} \quad (2)$$

Where Y_{ijk} is the k^{th} measurement subject to the i^{th} storage time and the j^{th} treatment, μ represents the general mean, τ_i is the effect of the storage time, α_j is the effect of the treatment, $\tau_i * \alpha_j$ represents the interaction between storage time i and treatment j, ε_{ijk} is the residual. The homoscedasticity was tested by the Breusch-Pagan test (Cook & Weisberg, 1983) and probability values below 0.05 were achieved, the model was analyzed via generalized linear models (GLM) (Pinheiro & Bates, 2000). The Bonferroni test was used for pairwise comparisons of the means. The relationship between the samples and the fatty acids was obtained by Principal Component Analysis (PCA), using the Pearson correlation matrix. The sensory data were treated by ANOVA considering treatments and storage time as factors and consumers as blocks. All statistical analyzes were performed in the R environment (R Core Team, 2016).

3. Results and discussion

Before making the description of the results and their corresponding discussions, a detailed analysis of the data was carried out (supplementary material). This section was divided into three parts: the first part is composed of the discussion of the headspace composition, microbiological analyzes, pH, and water holding capacity (Table 1). The second part includes the discussion of TPA (Table 2) and the last part the oxidative stability, trimethylamine formation, value K determination, and sensory analysis are described (Table 3).

The effects of modified atmosphere packaging (CT) in combination with chitosan (CF) films or with active chitosan films incorporated with pink pepper residue extract (CFPP) on the physicochemical, microbiological, and sensory characteristics of salmon fillets are presented in Tables 1-3. The columns show the significance level (P value) for the isolated effects and the interaction effects. For all the responses, the interaction effect was significant, suggesting the influence of storage time on treatment average response.

3.1. Total phenolic content in the pink pepper residue extract and active films

In the present study, the total phenolic content (PC) of agro-industrial residue of pink pepper was 29.2 ± 0.17 mg of gallic acid equivalents per gram of pink pepper residue. This value was higher than the one found by Romani, Hernández, and Martins (2018) - 12.03 mg of gallic acid equivalents per gram in pink pepper. On the contrary, our result was lower than that found by Serrano-León et al. (2018) - 45.01 mg of gallic acid equivalents per gram. Bergamachi (2016) evaluated the extraction of phenolic compounds from plants and concluded that the solvent ratio 80:20 v/v (ethyl alcohol: water) had the best extraction efficiency. This author obtained a content of 38.42 mg of gallic acid equivalents per gram of dry residue of pink pepper. Differences in PC results may be associated to many factors, such as climatic conditions, fruit variety, processing methods (extraction, filtration, insulation), and conditions of storage (air, temperature, time) (De Lima et al., 2017). The total phenolic content in the aliquot of extract incorporated in the filmogenic solution was 600 mg PC/L, by mixing one parts of pink pepper extract with four parts of filmogenic suspension. The concentration of total phenolic compounds in the CFPP film was 0.18 mg PC/cm^2.

3.2. Headspace composition

CO_2 concentration remained constant in the course of the storage period (Table 1). These results indicate that the air was efficiently removed from the package and replaced the atmosphere of the package by a volume of CO_2 (99.4–97.2%) enough to fully saturate the fish fillet, thus optimizing its bacteriostatic effect. This small decrease in CO_2 content may be related with the gas solubility in salmon fillets and with the permeability of the manufactured films (Fernández, Aspe, & Roeckel, 2009). To ensure the CO_2 balance around the product, the ratio of gas volume (L) to fish mass (kg) was approximately 5:1 (v/m).

3.3. Microbiological analysis

Microbial activity is mainly responsible for deterioration of seafood (Li, Li, Hu, & Li, 2013). No thermotolerant coliforms were detected in salmon samples and the initial counts of aerobic mesophilic and psychrotrophic microorganisms in salmon were 2.0 and 1.5 log CFU.g^{-1}, respectively, which indicates excellent microbiological quality, considering the limit of acceptability for 7 log CFU.g^{-1} of fresh fish (Ojagh, Rezaei, Razavi, & Hosseini, 2010).

It is evident that the use of 100% CO_2 inhibited the growth of bacteria, which allowed an extended storage period under refrigeration (Table 1). On the 14th day of storage, psychrotrophic bacteria counts reached 6.1 log CFU.g^{-1} for CT, while the CF and CFPP samples presented lower bacterial counts: 3.9 and 4.2 log CFU.g^{-1}, respectively (Table 1). The lactic bacteria showed the same trend, because on the 14th day of storage, the control sample had 5.8 log CFU.g^{-1}, while the samples with chitosan films showed much lower counts (CF – 2.7 log CFU.g^{-1} and CFPP – 2.8 log CFU.g^{-1}). It is evident, therefore, the chitosan films contributed to inhibit the multiplication of psychrotrophic and lactic bacteria, regardless of the incorporation of the pink pepper extract. In fact, some studies have shown that chitosan coating in fish fillets has antimicrobial effects against a wide range of microorganisms, improving the overall quality, and thus extending the product shelf life (Fan et al., 2009; Kostaki et al., 2009; Li et al., 2013). Overall, the pink pepper extract had no influence on the spoilage

Table 1
Microbial counts and physicochemical parameters of salmon fillets treatments during storage at 2 ± 1 °C.

Variable	Treatments	Storage time (days)			
		7	14	21	28
CO_2	CT	98.62 ± 0.16^{Aa}	98.36 ± 0.21^{ABa}	98.30 ± 0.21^{Ba}	98.40 ± 0.26^{ABa}
(%)	CF	98.46 ± 0.32^{Ab}	97.92 ± 0.61^{Ba}	98.68 ± 0.28^{ABb}	98.34 ± 0.17^{Bab}
	CFPP	98.66 ± 0.29^{Aa}	98.60 ± 0.07^{Aa}	98.90 ± 0.29^{Aa}	98.78 ± 0.33^{Aa}
TPVC	CT	3.08 ± 0.03^{Ac}	6.05 ± 0.00^{Ab}	6.35 ± 0.06^{Ab}	7.32 ± 0.09^{Aa}
(log CFU.g^{-1})	CF	2.45 ± 0.15^{Bd}	3.89 ± 0.11^{Bc}	5.60 ± 0.30^{Bb}	6.63 ± 0.00^{Ba}
	CFPP	3.31 ± 0.08^{Ad}	4.20 ± 0.12^{Bc}	5.87 ± 0.02^{Bb}	5.26 ± 0.08^{Ca}
LAB	CT	2.49 ± 0.04^{Ac}	5.78 ± 0.00^{Ab}	6.72 ± 0.06^{Aa}	7.05 ± 0.04^{Aa}
(log CFU.g^{-1})	CF	2.02 ± 0.02^{Bd}	2.66 ± 0.18^{Bc}	5.26 ± 0.01^{Cb}	6.27 ± 0.16^{Ba}
	CFPP	2.76 ± 0.02^{Ac}	2.75 ± 0.08^{Bc}	5.86 ± 0.04^{Bb}	6.28 ± 0.02^{Ba}
pH	CT	5.69 ± 0.11^{Cd}	6.02 ± 0.08^{Bc}	6.48 ± 0.09^{Bb}	5.87 ± 0.03^{Ba}
	CF	6.09 ± 0.05^{Bc}	5.87 ± 0.08^{Ab}	6.42 ± 0.03^{ABa}	5.89 ± 0.05^{Bb}
	CFPP	5.95 ± 0.05^{Ac}	6.06 ± 0.03^{Bb}	6.36 ± 0.11^{Aa}	5.99 ± 0.03^{Abc}
WHC	CT	72.53 ± 0.26^{Bd}	70.15 ± 0.54^{Bc}	67.72 ± 0.40^{Cb}	56.67 ± 0.31^{Ca}
(%)	CF	75.01 ± 0.57^{Ad}	72.27 ± 0.33^{Ac}	69.91 ± 0.62^{Bb}	64.93 ± 0.40^{Ba}
	CFPP	72.85 ± 0.61^{Bd}	70.26 ± 0.53^{Bc}	66.88 ± 0.47^{Ab}	64.40 ± 0.44^{Aa}

CT: Control; CF: Chitosan film; CFPP: Chitosan film incorporated with pink pepper residue extract.
TPVC: Total psychrotrophic viable count; LAB: Latic acid bacteria; WHC: Water holding capacity.
The results were expressed as mean ± standard deviation considering three independent replications for response. Different upper case letters indicate significant differences between the analyzed treatments (column), while different lowercase letters indicate significant differences between storage time (row), using Bonferroni test ($p < .05$).

microorganism counts during the storage period in relation to the chitosan film without pink pepper extract. Apparently, chitosan film trapped the active compounds avoiding its release towards the food (Souza et al., 2019). After 14 days, the differences of psychrotrophic and lactic acid counts in the CF and CFPP samples were lower in relation to the control sample. It can be observed that on the 28th day of storage, CF and CFPP samples had 6 log CFU.g^{-1} of psychrotrophic and lactic acid bacteria, while the control sample exceeded 7 log CFU.g^{-1} ($p < .05$) for these two groups (Ojagh et al., 2010). The number of spoilage bacteria may be indicative of the storage life of packaged products in modified atmosphere but may not be indicative of their sensory quality (Franco & Landgraf, 2008). As observed in the present study, sensory evaluation is an essential tool to monitor the storage life and safety of refrigerated salmon.

3.4. pH and WHC

Regarding the pH values, mean values between 5.7 and 6.5 were obtained (Table 1). All salmon fillets presented mean pH values within the limits required by current legislation (Júnior and de Oshiro, 2017) for fresh fish (pH ≤ 7.0). These values are similar to those reported for Atlantic salmon packed in a modified atmosphere (Milne & Powell, 2014; Sivertsvik, Rosnes, & Kleiberg, 2003). The oscillation in pH observed between treatments and between storage periods are associated with the production of basic amines (trimethylamine) through protein breakdown by the action of spoilage bacteria (Karabagias, Badeka, & Kontominas, 2011). A significant drop in pH values was observed after 21 days of storage for all treatments, probably due to the solubility of CO_2 in the free water of the fish muscle, producing carbonic acid and leading to its acidification (Velu, Abu Bakar, Mahyudin, Saari, & Zaman, 2013).

The WHC significantly decreased in the course of the refrigerated storage for all treatments. However, the CF and CFPP treatments showed the highest WHC on the 28th day of storage, differing significantly from CT (Table 1). A similar effect was reported elsewhere (Hultmann & Rustad, 2002; Lerfall, Bendiksen, Olsen, & Østerlie, 2015). The decrease in WHC observed in this study may be associated with both the solubilization of CO_2 in the muscle (Sivertsvik, Jeksrud, & Rosnes, 2002) and the structural changes in the muscle, such as proteolytic degradation of myofibrillar proteins and increased extracellular space (Kaale, Eikevik, Rustad, & Nordtvedt, 2014) caused by the bacterial growth.

3.5. TPA

TPA results are shown in Table 2. The hardness of all treatments

Table 2
TPA of salmon fillets treatments during storage at 2 ± 1 °C.

Variable	Treatments	Storage time (days)			
		7	14	21	28
Hardness	CT	1.75 ± 0.16^{Ac}	1.53 ± 0.06^{Bb}	1.28 ± 0.13^{Aa}	1.24 ± 0.03^{Aa}
	CF	1.80 ± 0.19^{Ab}	1.37 ± 0.05^{Aa}	1.35 ± 0.14^{Aa}	1.32 ± 0.09^{Aa}
	CFPP	1.80 ± 0.07^{Ab}	1.49 ± 0.08^{Ba}	1.40 ± 0.04^{Aa}	1.36 ± 0.14^{Aa}
Springiness	CT	0.44 ± 0.01^{Cb}	0.43 ± 0.03^{Bb}	0.37 ± 0.02^{Ba}	0.34 ± 0.02^{ABa}
	CF	0.42 ± 0.02^{Bb}	0.41 ± 0.02^{Bb}	0.36 ± 0.04^{Ba}	0.33 ± 0.02^{Ba}
	CFPP	0.45 ± 0.01^{Ac}	0.46 ± 0.02^{Ac}	0.43 ± 0.01^{Ab}	0.37 ± 0.04^{Aa}
Cohesiveness	CT	0.40 ± 0.05^{Bc}	0.27 ± 0.02^{Bb}	0.18 ± 0.02^{Ca}	0.17 ± 0.02^{Ca}
	CF	0.34 ± 0.01^{Ac}	0.25 ± 0.02^{Bb}	0.23 ± 0.03^{Bb}	0.15 ± 0.02^{Ba}
	CFPP	0.38 ± 0.03^{ABc}	0.18 ± 0.01^{Ab}	0.38 ± 0.01^{Ac}	0.34 ± 0.01^{Aa}

CT: Control; CF: Chitosan film; CFPP: Chitosan film incorporated with pink pepper residue extract.
The results were expressed as mean ± standard deviation considering three independent replications for response. Different upper case letters indicate significant differences between the analyzed treatments (column), while different lowercase letters indicate significant differences between storage time (row), using Bonferroni test ($p < .05$).

Table 3
Chemical and sensory parameters of salmon fillets treatments during storage at 2 ± 1 °C.

Variable	Treatments	Storage time (days)			
		7	14	21	28
TMA (mg.100 g^{-1})	CT	4.64 ± 0.31^{Ac}	5.76 ± 0.27^{Bb}	6.96 ± 0.38^{Ba}	7.18 ± 0.14^{Ba}
	CF	4.58 ± 0.31^{Ac}	6.66 ± 0.37^{Ab}	7.14 ± 0.11^{Ba}	7.19 ± 0.18^{Ba}
	CFPP	4.51 ± 0.30^{Ab}	6.40 ± 0.18^{Aa}	6.58 ± 0.22^{Aa}	6.61 ± 0.17^{Aa}
Value K (%)	CT	21.37 ± 0.40^{Cd}	25.07 ± 0.11^{Bc}	32.57 ± 0.03^{Cb}	46.41 ± 0.29^{Ca}
	CF	19.70 ± 0.41^{Bd}	21.60 ± 0.17^{Ac}	28.84 ± 0.21^{Bb}	37.20 ± 0.07^{Ba}
	CFPP	20.60 ± 0.03^{Ac}	21.08 ± 0.20^{Ac}	33.43 ± 0.18^{Ab}	38.76 ± 0.03^{Aa}
TBA (mg.Kg^{-1})	CT	0.22 ± 0.00^{Ab}	0.22 ± 0.00^{Ab}	0.22 ± 0.00^{Ab}	0.31 ± 0.07^{Ba}
	CF	0.22 ± 0.00^{Ab}	0.22 ± 0.00^{Ab}	0.22 ± 0.00^{Ab}	0.28 ± 0.01^{Aa}
	CFPP	0.22 ± 0.00^{Ab}	0.22 ± 0.00^{Ab}	0.22 ± 0.00^{Ab}	0.27 ± 0.01^{Aa}
Off-odor	CT	1.33 ± 0.97^{Ac}	2.44 ± 0.93^{Abc}	3.94 ± 1.47^{Ab}	4.18 ± 1.99^{Aa}
	CF	1.16 ± 1.09^{Ac}	2.02 ± 1.54^{Abc}	2.46 ± 1.91^{Ab}	4.06 ± 2.21^{Aa}
	CFPP	2.01 ± 2.33^{Ac}	2.06 ± 1.51^{Abc}	2.62 ± 1.75^{Ab}	3.76 ± 2.06^{Aa}
Overall appearance	CT	6.88 ± 0.96^{Aa}	5.83 ± 1.66^{Aa}	5.18 ± 1.36^{Aa}	4.58 ± 1.78^{Ab}
	CF	6.84 ± 1.36^{Aa}	6.44 ± 1.58^{Aa}	6.38 ± 1.62^{Aa}	4.83 ± 2.02^{Ab}
	CFPP	6.53 ± 2.24^{Aa}	6.26 ± 1.76^{Aa}	5.94 ± 1.56^{Aa}	4.94 ± 1.93^{Ab}

CT: Control; CF: Chitosan film; CFPP: Chitosan film incorporated with pink pepper residue extract.
TMA: Trimethylamine; TBA: Oxidative stability.
The results were expressed as mean ± standard deviation considering three independent replications for response. Different upper case letters indicate significant differences between the analyzed treatments (column), while different lowercase letters indicate significant differences between storage time (row), using Bonferroni test ($p < .05$).

decreased significantly during the storage period. Lower hardness values of salmon fillet during refrigerated storage were already reported in other studies (Hultmann & Rustad, 2002) and may be related to the decreased WHC (Wu & Sun, 2013). Muscle autolysis by endogenous and microbial proteolytic enzymes causes the breakdown of collagen binding resulting in texture changes (Chéret, Chapleau, Delbarre-Ladrat, Verrez-Bagnis, & de Lamballerie, 2005). Hardness of the fish muscle is a critical parameter that directly affects the acceptability of protein-rich foods (Mørkøre & Einen, 2003), being associated with both intrinsic factors (water content and distribution, fat content and distribution, collagen content) and extrinsic factors (time and temperature of storage and processing by which the fish is submitted) (Pearce, Rosenvold, Andersen, & Hopkins, 2011; Suárez, Abad, Ruiz-Cara, Estrada, & García-Gallego, 2005). Springiness of the samples allowed evaluating the percentage of recovery of the height of the samples between the first and the second compression. During storage, the springiness of all treatments decreased significantly. However, from the 14th day of storage, the CFPP samples presented higher springiness ($p < .05$) from the other treatments until the end of storage. The cohesiveness, which is a measure of the disintegration of the fillets, was significantly decreased in the course of storage. After 21 days, the salmon fillets packed in CFPP showed significantly higher cohesiveness than the other treatments. Probably, the reduction of elasticity and cohesiveness of salmon fillets during refrigerated storage is associated to the degradation of high molecular weight proteins, as previously reported by Hultmann and Rustad (2002).

3.6. Instrumental color

Color is an important quality parameter for appearance of fresh salmon. The color parameters (Fig. 1) showed significant interaction ($p < .05$) between treatment and storage time. CFPP and CF salmon were found to be darker (lower L^*-value), more reddish (higher a^*-value), and more yellowish (higher b^*-value) as compared to the CT treatment, after 28 days of storage ($p < .05$). Higher a* values are related to the red-orange or reddish color in fresh salmon caused by carotenoids (astaxanthin and cataxanthines). Similar results for reddish color has been reported in fresh salmon by Dias et al. (2019). The reddish color in fresh salmon is associated with market acceptance and receives different prices depending on perceived color (Lerfall et al., 2015). The chitosan film used, even without pink pepper, was efficient to minimize the oxidation of pigments of salmon. The simple use of anoxic atmosphere (CT) aids to prevent color oxidation. Ottestad, Sørheim, Heia, Skaret, and Wold (2011) observed significant differences in a* values between all atmospheres tested (CO_2 > vacuum > air), suggesting that the effect of the heme pigment on salmon color can be masked by the light absorption of carotenoids. Changes in L* values after rigor mortis are related to changes in the surface muscle light scattering (Erikson, Shabani, Beli, Muji, & Rexhepi, 2018). Myofibrillar, cytoskeletal and interaction with other sarcoplasmic proteins can both absorb and scatter the light and affect the paleness/darkness of the fish muscle (Hughes, Clarke, Li, Purslow, & Warner, 2019). In this study, structural changes of muscle proteins increase the light scatter and the lightening (L*) of the surface salmon fillet over time, indicating an opaque color. These structural changes were more evident in the CT samples, which exhibited a greater muscle proteolysis (lower WHC and lower hardness) and, consequently, greater light scattering and higher L* values at the end of storage. In parallel, the lipid auto-oxidation products, the aldehydes, can interact with the amino groups of proteins and lead to the development of yellowish (higher b^*-value) color (Zhang, Xiao, & Ahn, 2013).

3.7. Trimethylamine (TMA)

TMA is produced from trimethyl amine oxide (TMAO) that acts as an oxygen donor for bacterial growth in some seafood products (Giménez, Roncalés, & Beltrán, 2005). In Table 3, the increase of TMA for all treatments during storage is shown. The salmon samples of all treatments presented lower TMA values compared to the limits established by the European Community: 12 mg TMA per 100 g of sample (Mohan, Ravishankar, Lalitha, & Srinivasa Gopal, 2012). However, up the 28th day of storage, the lowest TMA values ($p < .05$) were found in salmon fillets packed with chitosan films containing pink pepper residue extract (CFPP). The highest TMA values found for the control treatment are associated to the high CO_2 concentration. Indeed, CO_2 has an inhibitory effect on the growth of aerobic deteriorating bacteria but presents a limited effect on TMA production (Jérôme, Macé, Dousset, Pot, & Joffraud, 2016). Similar results were found by Souza et al. (2010), who observed values of 6.37 ± 0.14 mg TMA per 100 g in uncoated salmon fillets and 5.33 ± 0.29 mg TMA per 100 g in chitosan-coated salmon fillets, after 18 days of storage at 0 °C. These results reinforce the hypothesis of a synergistic effect between the modified

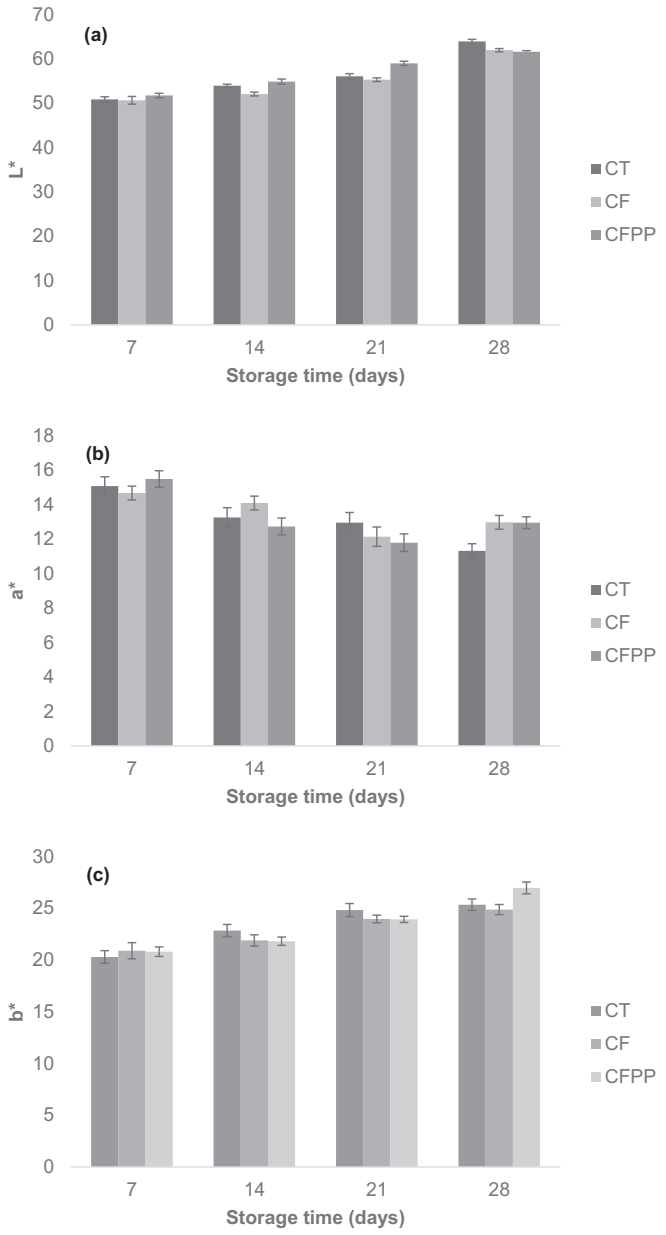

Fig. 1. Instrumental color (L*, a*, b*) during refrigerated storage (2 ± 1 °C) of salmon fillets. The results are the mean of three replications and the bars indicate the standard deviation. CT: control treatment (100% CO_2); CF: chitosan films and 100% CO_2; and CFPP: chitosan films incorporated with pink pepper residue extract and 100% CO_2. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

atmosphere and the bioactive compounds of pink pepper extract may retard the growth of Gram-negative bacteria that perform oxidative deamination (Li et al., 2013; Souza et al., 2010).

3.8. Oxidative stability

The TBA value of CT treatment was higher ($p < .05$) than the values found for CFPP and CF on the 28th day of storage (Table 3), while on the other days the TBA values were similar for all treatments. These results suggest that the anoxic atmosphere used for packaging may contribute to reduce lipid oxidation. Successful of modified atmosphere packaging is related to the gas volume/product ratio used for different products (Sivertsvik, Rosnes, & Jeksrud, 2004) and the antioxidant activity of polyphenols from the pink pepper residue extract incorporated in the chitosan film did not increase the antioxidant ability of chitosan in MAP storage. The protection against oxidation may depend on the type antioxidant found in plants species and also of their concentrations (Fang, Lin, Warner, & Ha, 2018).

The establishment of TBA values is not easy to be made because internal and external factors can influence such values. Thus, the limits values for TBA in fish are controversial. Some authors indicate that values of 1 to 2 mg of MDA per kilogram of fish may be considered as a limit beyond which fish may develop an unpleasant odor. While, other authors indicate that values of 5 to 8 mg of MDA may indicate that the fish is of good quality and can be consumed (Giménez et al., 2005; Sallam, 2007).

The values found are much lower than the reported limits by Giménez et al. (2005), who found TBARS around 1 mg malonaldehyde per kilogram for salmon fillet added with natural antioxidants and packaged in modified atmosphere (50% CO_2 and 50% N_2) after 20 days storage at 1 °C. And, the TBA values are similar than the reported by Gómez-Estaca et al. (2018) for salmon carpaccio covered with chitosan edible film incorporated with clove essential oil. In this way, the low TBA values can be attributed to the antioxidant effect of active compounds incorporated in chitosan films (Giménez, Gómez-Guillén, López-Caballero, Gómez-Estaca, & Montero, 2012). It may therefore be concluded that the TBA values were much smaller than the limits considered the threshold value for the perception of lipid oxidation by consumers, being in agreement with the results of the sensory analysis of the present study.

3.9. Value K (%)

Value K (%) is used to evaluate the freshness of fish and the analysis is based on the concentration of compounds produced from the degradation of adenosine triphosphate (ATP) due to endogenous enzymatic activity (Nollet & Toldrá, 2010). The concentration of adenine nucleotides may be affected during storage by several factors, such as fish species, type of muscle analyzed and storage conditions (Li et al., 2013). Analyzing the effect of storage time, for all treatments, there was a significant increase ($p < .05$) in the value K (Table 3). In general, the value K of CF and CFPP were significantly lower compared to CT, except for the 21st day. Similar values K were reported for chitosan-coated silver carp during frozen storage for 30 days (Fan et al., 2009). Li et al. (2012) and Fan et al. (2009) also attributed that the lowest nucleotide degradation in the samples packed with bioactive films is probably related to the inhibition of nucleotidase enzyme activity, which is responsible for the decomposition of inosine monophosphate (IMP) by chitosan.

3.10. Fatty acid profile

The first two principal components of PCA accounted for 84.15% of the original variability (Fig. 2). A clear differentiation was observed between treatments after 28 days of storage. Fish species with a high fat content, such as salmon, are good sources of PUFAs, especially omega-3 fatty acids (Dave & Routray, 2018). At day 0 (CT-0), the monounsaturated fatty acids (C18:1 and C22:1) were the major acids in the salmon fillets. However, after 28 days of storage, salmon fillets packed with active chitosan films (CFPP-28) were characterized by a higher amount of essential PUFAs when compared to the CF-28 and CT-28 samples, such as linolenic acid (C18:3), linoleic acid (C18:2) and docosahexaenoic acid (DHA, C22:6). The antioxidant capacity of pink pepper extract may have prevented the oxidation of polyunsaturated fatty acids during storage. This study suggests that the incorporation of pink pepper extract into the chitosan film (CFPP) potentiated the antioxidant capacity compared to the chitosan (CF) film in the anoxic (oxygen free) atmosphere (Pasanphan, Buettner, & Chirachanchai, 2010).

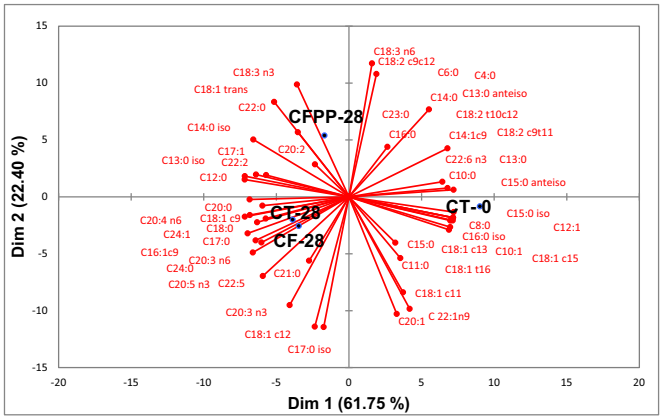

Fig. 2. Fatty acids composition present in Atlantic Salmon fillets (*Salmo salar*) stored at 2 ± 1 °C in different treatments (CT: control treatment with 100% CO_2; CF: chitosan films with 100% CO_2; and CFPP: chitosan films incorporated with pink pepper residue extract and 100% CO_2). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.11. Sensory analysis

The sensory evaluation showed a significant decrease in acceptance in all samples in the course of storage time (Table 3). The panelists noticed an increase of unpleasant off-odor during the storage period, but they did not indicate the rancid odor. Rather, from the 21st day of storage onwards, panelists noticed acid and/or mild ammonia odors. These changes may be related to i) the growth of lactic acid bacteria that is favored by modified atmosphere with high concentration of CO_2 (Karabagias et al., 2011) and ii) to the increase of oxidative deamination as a function of the bacterial growth in salmon fillets (Françoise, 2010). Similar results were reported by Hansen et al. (2009) who also observed increased acid and ammonia odors in Atlantic salmon packed in a high CO_2, modified atmosphere after 15 days of refrigerated storage.

Compared with control treatment, the overall appearance of salmon fillets was not significantly affected by the odor and neither by the presence of chitosan film and chitosan film manufactured with pink pepper residue extract. Other authors (Li et al., 2012; Li et al., 2013) concluded that fish treated with chitosan films and natural preservatives presented better or similar sensory characteristics than the control treatment, which further supports our results.

4. Conclusion

Biodegradable absorbent pad from chitosan with the incorporation of natural substance was the most successful treatment to replace synthetic absorbents pads in the modified atmosphere packaging system for salmon fillets. Chitosan films with pink pepper residue extract (CFPP) showed higher antibacterial activity when compared to chitosan films (CF) or with synthetic absorbent pads (CT). For this reason, it was the best treatment for maintaining the firm structure of fillets (lower proteolysis, higher elasticity and cohesiveness) and for the prevention of TMA formation caused by Gram-negative bacteria. CFPP also showed high antioxidant activity and, consequently, higher color stability. Despite the satisfactory results of CFPP for fresh fish preservation purposes, further studies are needed to evaluate the impact of incorporating pink pepper residue extract into biodegradable absorbents in concerning to acceptability of this packaging system by consumers, before commercial application.

Declaration of Competing Interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodres.2019.108633.

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