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Efficacy of an Aromatic Vinegar in Reducing Psychrotrophic Bacteria and Biogenic Amines in Salmon Fillets (*Salmo salar*) Stored in Modified Atmosphere Packaging

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Salmon flesh spoilage can be greatly reduced through the use of preservation methods, using natural products combined with low temperature and packaging. Microbiological and physicochemical characteristics of fresh salmon fillets (*Salmo salar)*, sprayed with an aromatic vinegar and stored in modified atmosphere packaging, were investigated. Fillets were kept at 4°C and sampled after 2 h and 3, 7 and 10 days. An untreated control group was used as well. Fish samples were analysed for microbiological (total viable count, *Enterobacteriaceae*, psychrotrophic microbial count, *Pseudomonas* spp.) and physicochemical (pH, colour, total volatile basic nitrogen, and biogenic amines) properties. Aromatic vinegar was found to beneficially contribute to the hygienic quality of the salmon, reducing microbial growth during storage and exerting a positive effect, mainly on psychrotrophic loads and *Pseudomonas* spp. The treatment had a positive effect on biogenic amine levels, showing lower values for histamine, putrescine, cadaverine, and tyramine.

INTRODUCTION

In the Western world, people are paying more attention to healthy eating; as a result of which seafood, including fish, crustaceans, molluscs and edible aquatic plants, is in high demand as an important source of omega 3 fatty acids [Branciari *et al*., 2017; Leisner & Gram, 2014; Tacon & Metian, 2013]. Aquaculture is the fastest growing food sector worldwide, and among seafood, farmed salmon is one of the most highly valued products [Briones *et al*., 2010; FAO, 2016], from both the nutritional and the economic perspectives [Sivertsvik *et al*., 2003]. Aquaculturists face many challenges to meet the global food demand while maintaining a high-quality product. Due to its high nutritional quality, fresh salmon is popular and is consumed throughout the world; however, it is also prone to spoilage [Wang *et al*., 2017a]. Food structure, together with chemical composition and storage conditions (temperature, time, packaging system, light, and antimicrobials), strongly affect the growth and proliferation of microorganisms [Corbo *et al*., 2009]. Indeed, fish is one of the most perishable food products [Fidalgo *et al*., 2018] due to the presence of non-protein nitrogenous substances, lipid composition, neutral pH and a high moisture content which are suitable conditions for microbial proliferation [Miraglia *et al*., 2016]. Microbial growth and activity are primarily responsible for the development of off-odours and off-flavours that make non-frozen fish products unacceptable or spoiled [Leisner & Gram, 2014] and can pose a significant threat to the health of consumers [Ozogul *et al*., 2017].

Market needs, added to consumer demand for fresh, refrigerated, minimally-processed and long-life food, have led to a considerable research effort aimed to define new technologies able to preserve texture, flavour and nutritional value and to ensure fresh fishery products' safety and quality [Fernández *et al*., 2009; Miks-Krajnik *et al*., 2016; Sallam, 2007]. Various methods of preservation have been assessed, investigating their effects on water activity, pH, low temperature or modified atmosphere packaging. These parameters have been shown to have a great effect on the microbial flora of fish and on the corresponding spoilage pattern [Leisner & Gram, 2014]. Green consumerism, that is the growing demand for fewer chemical preservatives, environmentally friendly antimicrobials and the sustainable use of molecules of natural origin [Corbo *et al*., 2009], together with the demand for high-quality fresh seafood, has intensified the search for technologies that favour fresh fish utilisation. Nevertheless, effects of modified atmosphere packaging (MAP) of fresh fish are controversial with author who report

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limited extension [Emborg *et al*., 2002] or an improvement of fish shelf life but in relation to fish species, initial microbial load, gas mixture, and temperature of storage [Emborg *et al*., 2002]. Among natural products, vinegar shows a significant biological activity, including sanitising characteristics and antioxidant effects [Lingham *et al*., 2012; Machado *et al*., 2011]. It is recognised as a natural antimicrobial and antioxidant product that may improve safety and enhance shelf life, provide acceptable sensory quality and reduce economic losses due to spoilage of fish products [Lingham *et al*., 2012].

In view of what has been said regarding the importance of fish from a nutritional, qualitative, and economic standpoint, the purpose of this study was to investigate the effects of surface treatment with an aromatic vinegar on some microbiological and physicochemical parameters related to the quality and safety of salmon fillets stored in MAP, and thus to reduce the bacterial populations, to determine changes in biogenic amines of the fish, which are beneficial for producer interest and consumer safety.

MATERIALS AND METHODS

Experimental design

Chilled fillets of salmon (*Salmo salar*) and aromatic vinegar (AV) obtained from sugar cane (Aromatic Vinegar GPI 6.2® – Lazzari Equipment & Packaging, Settimo di Pescantina, VR, Italy) were purchased from a local factory (Circeo Pesca S.r.l., San Mariano-Corciano, PG, Italy). Fillets were obtained seven days after harvesting from salmon weighing between 3 and 4 kg, stored in ice till filleting. The fillets were then divided in 150 g portions (12 g standard deviation) presenting both side (inner and outer part) and ventral and dorsal parts (surface of 90 cm^2 approximately). The skin was left on the outer side according to producer needs.

In order to perform analytical determinations of AV, on the first day of the trial, 100 mL of the product were sampled in triplicate from each of three vats containing 23 L of AV. Salmon fillets (150 g each) were treated by spraying homogeneously their inner surface with 1.5 mL AV/150 g of product by a hand nebulizer and subsequently packaged on polystyrene trays (Sirap-Gema S.p.A., Verolanuova, Italy) under MAP (Delta 2000; Ilapak Italia S.p.A. Foiano della Chiana, Italy) and covered with a stretch-film (Cryovac® BDFS100; thickness = 21 μm; density = 0.943 g/cm^3; Permeability: CO^2 = 100 cm^3/m^2, 24h; O^2 = 25 cm^3/m^2, 24h); they were referred to as the AV group. Untreated salmon fillets, stored at the same conditions as AV samples, were employed as a control group (C group). The gas content of MAP was measured using a CheckPoint Handheld Gas Analyser (PBI Dansensor, Ringsted, Denmark), and O_2 and CO_2 ranged from 13.46% and 28.22% on day 0 to 13.92% and 16.52% on day 10 of storage, respectively.

All fish samples were kept in the dark during storage under refrigeration (4±1°C) directly at factory level and were collected after 2 h (T1), 3 days (T3), 7 days (T7) (shelf life of the salmon fillets defined by the producer), and 10 days (T10). At each sampling point, a total of 20 samples were used, 10 each for both AV and C groups. All the fillets and AV samples were transported in refrigerated conditions (4±1°C) to the laboratory at the Department of Veterinary Medicine (University of Perugia, Italy) and promptly processed for microbiological and physicochemical analysis.

Physical-chemical determination of the aromatic vinegar

Vinegar samples were analysed for pH value, using a pH meter equipped with an insertion electrode (Crison pH25, Crison, Barcelona, Spain). Total polyphenols content (TPC) was determined using the Folin–Ciocalteu colorimetric method [Rashidinejad *et al*., 2013] using an Ultrospec 2100 pro UV/visible spectrometer (Amersham Pharmacia Biotech, Buckinghamshire, UK) at 765 nm. The phenolic content was calculated based on a gallic acid calibration curve as reported in Miraglia *et al*. [2017] ($y = 0.001x - 0.0273$, $R^2 = 0.9943$). The content was expressed as mg gallic acid equivalents (GAE) per mL. No microbiological analyses were performed for vinegar, considering the characteristic of the products, and the product sheet reports (Lazzari Equipment & Packaging, Settimo di Pescantina, VR, Italy): total bacterial count < 5000 colony-forming units (CFU)/g; yeasts and moulds < 300 CFU/g; *Salmonella* spp., *Listeria monocytogenes*, *Escherichia coli*, and *Staphylococcus aureus* absent in 25 g.

Microbiological analysis of salmon fillets

For sample preparation, a portion of 10 g (±0.1 g) from the upper left side of each salmon fillet was sampled using aseptic blades and forceps and was transferred into a sterile stomacher bag with 90 mL of sterile peptone water. After homogenisation (Stomacher 400 circulator; Seward Ltd, Norfolk, UK), the following microbial counts were determined: total viable count (TVC) according to ISO 4833–1:2013; *Enterobacteriaceae* count according to ISO 21528–2:2017; psychrotrophic microbial count according to ISO 17410:2001; and *Pseudomonas* spp. count by the spread method: 0.1 mL was incubated in Pseudomonas CFC Selective Agar Base (Biolife, Milan, Italy) plates, that were incubated at 25°C for 24 h in aerobic conditions. Results of microbial analyses are expressed as log CFU/g.

Salmonella spp. detection was performed according to ISO 6579–1:2017, and *L. monocytogenes* presence was investigated according to ISO 11290–1:2017.

Physical-chemical determinations of salmon fillets

Colour was evaluated on the cut surface of the fillet with a colorimeter (Minolta CR 400, Osaka, Japan) using the CIE L* a* b* system [CIE, 1986], and the measurements were performed in duplicate on the dorsal and ventral part of each fillet. Results are expressed as mean values of four measurements. The pH value of each salmon fillet was determined in the right dorsal part of the fillets in triplicate using a pH meter equipped with an insertion probe (Crison 25, Crison, Barcelona, Spain). Water activity (a_w) was determined using a HygroLab 3 hygrometer (Rotronic, Huntington, NY, USA) on three samples collected from the upper right part of the fillets. Total volatile basic nitrogen (TVB-N) content was determined in duplicate at the lower right part of the fillets according to Pearson [1991].

Content of biogenic amines (BA) was determined in eight samples for each treatment at each sampling point considered. BA extraction from the samples was carried out according to

the procedures developed by Zhai *et al*. [2012] with a little modification. Five grams of each sample was transferred into a centrifuge tube containing 5% (w/v) trichloroacetic acid (TCA). The mixture was vortexed and centrifuged at 10,000 × *g* for 5 min at 4°C. The supernatant was collected, and the residue was extracted again with the same volume of TCA. Both supernatants were filtered through Whatman paper No. 1 and combined. The final volume was adjusted to 25 mL with TCA. BA derivatization and quantification was carried out according to the procedures described by Zhai *et al*. [2012]; the quantification was performed using high--performance liquid chromatography (HPLC) analysis using a Shimadzu RF-20AXS instrument (Kyoto, Japan) consisting of a vacuum degasser, a quaternary pump, an autosampler, a thermostatted column compartment, and a fluorescent detector. Histamine (HIS, 98%), tryptamine (TRP, 98%), cadaverine (CAD, 98%) and 2-phenylethylamine (2-PHE, 99.5%) were purchased as standards from Sigma Aldrich Italia (Milan, Italy); whereas spermine (SPM, 97%), tyramine (TYR, 99%), putrescine (PUT, 98%), and spermidine (SPD, 98%) were purchased as hydrochloride salts (Sigma Aldrich). Results were expressed as mg/kg.

Statistical analysis

The data of the different parameters considered were analysed by an ANOVA model (SAS Institute Inc., Cary, NC, USA) with treatment (AV, C) and time (T1, T3, T7, and T10) as fixed and variable factors, respectively, and their interactions. The ComBase software tool DMfit (Baranyi and Roberts model) was used to determine growth curves for all the microbial populations considered in AV and C samples. The results of the model, expressed as initial value, shoulder, μ max and final value, were analysed by a one-way ANOVA model (SAS Institute Inc., Cary, NC, USA). For all these parameters, the difference between the means was analysed with the Tukey test and considered significant at a p value <0.05.

RESULTS AND DISCUSSION

pH and TPC of aromatic vinegar

In order to assess the peculiar characteristics of the treatment, pH and TPC analyses were performed in the AV. The average pH value recorded was 6.20 (0.08 standard deviation), and the TPC value was 2.6 mg GAE/mL (0.2 standard deviation). The pH was exactly comparable to that reported in the product sheet, and relatively high compared to other vinegars reported in the literature as antimicrobials [Medina *et al*., 2007]. The TPC was comparable to that reported in other vinegars [Machado *et al*., 2011] but lower than those found in other natural compounds adopted in fish fillets and reported in the literature [Choulitoudi *et al*., 2016].

Salmon fillet microbiology

The microbial loads of fish fillets are shown in Figure 1. In order to assess whether the use of AV had any effect on bacterial growth in salmon fillets, microbiological data were analysed using ComBase, and the microbial responses are reported in Table 1. The parameters considered were initial value (log CFU/g), lag/shoulder (h), μ max (Log CFU/g/h), and final value (log CFU/g) [Baranyi & Tamplin, 2004].

Counts of all bacterial groups increased throughout the storage experiment at chilled temperature, showing initial counts between 10^2 and 10^4 CFU/g (Table 1). Salmon fillets were considered to be in good condition for human consumption before the storage experiment, as the initial contamination level for raw fish at time zero should be below 10^6 CFU/g of TVC as reported by Miks-Krajnik *et al*. [2016]. Initial TVC was between 4.35 and 4.59 Log CFU/g for the two groups, which was slightly lower than reported by Fidalgo *et al*. [2018] but in accordance with values reported by Chytiri *et al*. [2004]. The final TVC values were lower than those reported in the literature that reached 8 log CFU/g after 6 days of storage [Fidalgo *et al*., 2018; Miraglia *et al*., 2016]. No differences in the final TVC were recorded; therefore, it was shown that the treatment had no effect on TVC.

Initial *Enterobacteriaceae* loads varied between 2.37 and 2.50 log CFU/g, which is in accordance with Chytiri *et al*. [2004] and Fidalgo *et al*. [2018]. After 10 days, loads reached about 4.95 CFU/g in control samples, showing a similar pattern to those reported by Chytiri *et al*. [2004]. Even though the *Enterobacteriaceae* count showed consistent contamination in the two groups, and treated samples at T10 showed a slight decrease in bacterial loads, there was no significant difference due to the treatment. In addition,

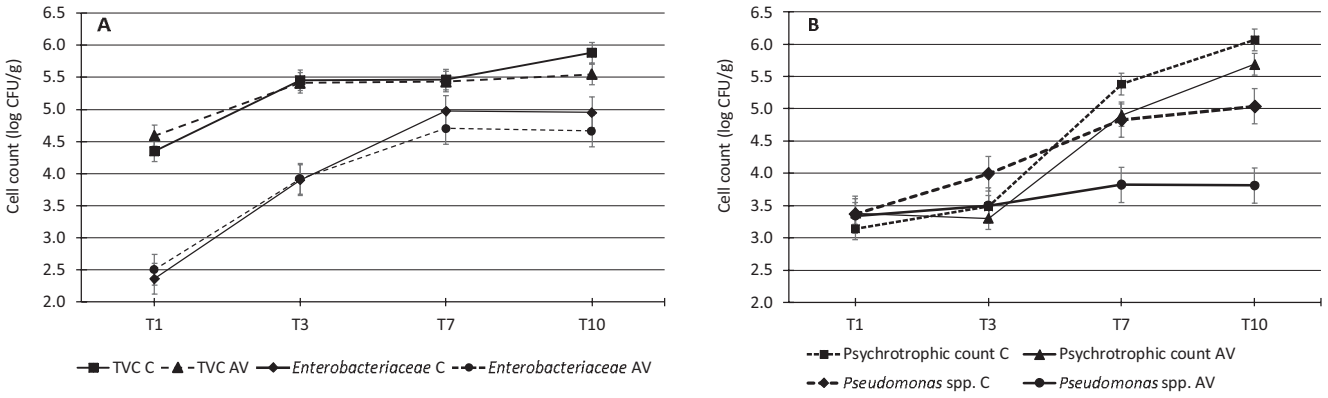

FIGURE 1. Total viable count (TVC), and *Enterobacteriaceae* (A), psychrotrophic and *Pseudomonas* spp. counts (B) of the salmon fillets throughout the storage time (T1 – 2h, T3 – 3 days, T7 – 7 days, T10 – 10 days). C – control group; AV – salmon fillets treated with aromatic vinegar.

TABLE 1. Effects of the aromatic vinegar addition to salmon fillets on kinetic parameters describing the growth of bacterial loads performed (following Baranyi–Roberts models).

Kinetic parameters of growth	Treatment	TVC	*Enterobacteriaceae*	Psychrophilic count	*Pseudomonas* spp.
Initial value (log CFU/g)	C	3.93	1.98	2.74	3.21
	AV	3.93	2.27	3.13	3.41
Lag/shoulder (h)	C	17.15	16.95	39.07^{a}	25.37
	AV	14.77	35.12	77.25^{b}	60.92
μ max (log CFU/g/h)	C	0.0294	0.0318	0.0372	0.0198^{b}
	AV	0.0264	0.0306	0.0198	0.0048^{a}
Final value (log CFU/g)	C	6.30	4.99	6.77^{b}	5.50^{b}
	AV	6.20	4.89	6.12^{a}	3.96^{a}

Within each microbial load, different superscript letters (a, b) indicate differences between treatments ($p \leq 0.05$). TVC – total viable count, C – control group, AV – salmon fillets with aromatic vinegar.

neither *Salmonella* spp. nor *L. monocytogenes* were detected in any of the samples tested.

The initial psychrotrophic load of control samples was 3.14 log CFU/g, similarly to that reported by Briones *et al*. [2010]. A limit of 10^5 CFU/g for psychrotrophic bacteria has been suggested for fresh fish [Pons-Sànchez-Cascado *et al*., 2006], and this limit was exceeded after 7 and 10 days of storage for control and treated samples, respectively. A significant difference between treated and control samples was detected at T3 (7 days), considered the end of commercial shelf life for this kind of product. As shown in Table 1, the addition of the aromatic vinegar seems to influence the growth dynamics of psychrotrophic bacteria through extension of the lag phase (λ) which also results in a lower final value. The initial *Pseudomonas* count showed values between 3.37 and 4.04 log CFU/g, *i.e.* slightly lower than those reported by Fidalgo *et al*. [2018] and in accordance with the loads reported by Chytiri *et al*. [2004] for filleted rainbow trout. However, in their study, a count of approximately 7 log CFU/g was reached for fillets after 10–11 days of storage, which was considerably higher than the maximum of 4.04 log CFU/g found in this work. Furthermore, *Pseudomonas* counts reported the most relevant differences as there was a significant difference between groups at both T3 and T10. The AV affected both their μ max and final values.

Although the specific mechanisms of action have not been completely clarified, it has already been reported in the literature that, within a specific matrix, natural compounds (such as R (+) limonene, oregano, thyme, and star anise essential oils) may act differently on different bacteria [Giarratana *et al*., 2016; Huang *et al*., 2018], mainly due to the specific microbial response. The effect of vinegars on microbial population are depending on both organic acids and polyphenols [Bakir *et al*., 2017; Chen *et al*., 2016]. As regards organic acids, the main mechanism of action proposed involves the acidification of the bacterial cytoplasm after the compounds penetrated the cell membranes, and is not merely a question of the pH value of the solution adopted [Bakir *et al*., 2017; Kundukad *et al*., 2017]. The lower pKa of the organic acids than the pH of the cytoplasm causes their dissociation into hydrogen ions, the rise of the acidity inside the cell and damages to both structural and functional proteins [Yagnik *et al*., 2018]. Furthermore, to counteract hydrogen ions concentration, a high quantity of energy is needed thus limiting the macromolecular synthesis and microbial growth [Van Immerseel *et al*., 2006]. Other proposed mechanisms of organic acids action are the increasing osmotic pressure and the production of antimicrobial peptides inside the cell [Chen *et al*., 2016]. Polyphenols either exert antimicrobial effects, depending on compounds structure and their concentration, through cell membrane disruption, interference with bacterial cell enzymes, and chelation of essential metals after they had penetrated the bacterial cell membrane [Chen *et al*., 2016; Daglia, 2012].

As mentioned before, for psychrotrophic bacteria, the AV inhibits the early steps of microbial growth and therefore influences the final concentration at the last sampling time, while for *Pseudomonas* spp., the effects are due to growth inhibition during the exponential phase.

Overall, the treatment had a positive effect, mainly on the inhibition of psychrotrophic bacteria and *Pseudomonas* spp. counts in salmon fillets, while no significant effect was observed on either TVC or *Enterobacteriaceae* count.

The microflora present in fish depends on the species, habitat, environment, harvesting method, and storage conditions and determines which bacteria are responsible for spoilage [Briones *et al*., 2010]. It is important to establish the count of *Pseudomonas* spp. as it represents the specific spoilage organism in freshwater fish [Gram & Dalgaard, 2002]. *Enterobacteriaceae* count is a hygiene indicator [Mexis *et al*., 2009], and different enteric species of histamine-producing bacteria have also been isolated from fish. Moreover, *Pseudomonas* spp. have also been reported as histamine producers [Hu *et al*., 2014; Wang *et al*., 2017b].

The definition of a threshold value for spoilage microbial growth depends on the product and the microorganism considered. For sea products, especially fish, some studies report that level of specific spoilage flora, such as *Pseudomonads* and *Enterobacteriaceae*, accounting for 10^7 CFU/g coincides with the appearance of irreversible alterations, therefore determining the end of the shelf-life [ICMSF, 1986; Koutsoumanis, 2001; Koutsoumanis & Nychas 2000]. In the present

study, the mentioned value was never reached in neither experimental group. Accordingly, the fillets remained at an acceptable standard for consumption throughout the storage.

Biogenic amines (Bas) in salmon fillets

BAs are non-volatile basic compounds formed by decarboxylation of the precursor amino acids as a result of metabolic processes of bacterial enzymes and are absent or present at very low levels in fresh fish [Chytiri *et al*., 2004; Pons-Sànchez-Cascado *et al*., 2006]. BAs are of importance due to food intoxication risk as they cause the most common food poisoning associated with fish consumption [Kim *et al*., 2009; Ozogul *et al*., 2017], and, although their formation in food does not necessarily correlate with the growth of spoilage organisms, they are useful chemical indicators of spoilage and thus of loss of freshness, hygienic quality of fish, and consumer acceptance [Kim *et al*., 2009; Pons-Sànchez-Cascado *et al*., 2006; Santos, 1996; Wunderlichová *et al*., 2014]. Although dominant microbial groups and concentrations in a fish species vary with temperature and storage conditions, affecting the formation of specific amines in the muscle [Chytiri *et al*., 2004], few studies have related bacterial and sensorial changes to BAs formation [Emborg *et al*., 2002]. Furthermore, *Salmonidae* have been regularly reported to cause histamine fish poisoning [Ozogul *et al*., 2017].

In this study, the presence of eight BAs was assessed but only seven were detected, namely 2-phenylethylamine, putrescine, cadaverine, histamine, tyramine, spermidine, and spermine; while tryptamine was not detected. Histamine content was the highest, reaching a final value of 59 mg/kg in the control samples after 10 days, whereas it was not detected in the treated samples. Thus, the aromatic vinegar exerted a positive effect on histamine production (Table 2). The European Community established a value for histamine at 200 mg/kg in fish [Regulation (EC) No 1019/2013] and the Food and Drug Administration (FDA) allows a lower level (50 mg/kg) [FDA, 2011]. This value was surpassed after 10 days of storage in the control samples.

In the control samples, contents of putrescine, cadaverine, and histamine increased steadily between days 7 (T2) and 10 (T3). Sensorial and microbiological data showed that 13 mg/kg of putrescine is the upper limit for spoilage initiation in fresh rainbow trout fillets [Chytiri *et al*., 2004]. This value was achieved at T10 in the control samples, but not in the AV group. The elevated content of histamine along with those of putrescine and cadaverine depicts bacterial spoilage of fish tissue. Indeed, the count of certain microbial groups is relevant, such as enterobacteria and pseudomonads that have been described as active BA-producing microorganisms in fish [Pons-Sànchez-Cascado *et al*., 2006]. As it has been reported by Chytiri *et al*. [2004], *Pseudomonas* spp. can lead to the synthesis of putrescine and cadaverine, as they are responsible for the carboxylation of lysine and ornithine, respectively. Moreover, putrescine and cadaverine can interfere with the histamine detoxification system, enhancing the toxicity of histamine [Kim *et al*., 2009].

Moreover, there was a significant difference between the control and treatment groups in tyramine levels throughout the storage time, with values established for the treatment groups being much lower than these determined for the control (Table 2).

pH, a_w, colour and TVB-N in salmon fillets

The effect of the treatment and storage time on the pH value of salmon fillets is shown in Table 3. The initial pH value of the control and treated fillets was 6.13, which is in accordance with the mean cut-surface pH at time zero reported by Fletcher *et al*. [2002] and by Emborg *et al*. [2002]. During the study, pH remained stable, and only a slight decrease was reported after 3 days of storage, to 5.97 and 6.08 for AV and C groups, respectively. The pH values of salmon flesh decrease *post mortem* because of lactic acid formation, while increases are usually observed due to the production of basic nitrogen from bacterial growth [Briones *et al*., 2010]. Although there was an increase in the production of nitrogenous compounds during the storage period due to microbial growth, the pH did

TABLE 2. Biogenic amine content in salmon fillets, control and treated with aromatic vinegar (mg/kg), throughout the storage time.

Treatment	Time	2-PHE	PUT	CAD	HIS	TYR	SPD	SPM
C	T1	34.87±2.10	8.62±5.45	–	–	28.25±5.01^{b}	5.87±2.70	25.75±3.49
	T3	32.62±4.34	6.25±4.46	–	–	25.88±5.17^{b}	6.00±2.39	24.25±2.87
	T7	32.12±5.43	8.25±5.44	10.12±4.19^{bx}	8.25±3.79^{x}	24.13±4.05^{b}	5.75±2.82	23.38±2.96
	T10	34.62±5.04	12.87±8.63^{b}	45.13±14.94 ^{by}	58.63±13.56^{y}	26.00±4.17^{b}	5.62±2.13	20.37±3.42
AV	T1	32.87±2.42	5.37±3.85	–	–	7.38±4.27^{a}	5.12±2.23	22.12±2.70
	T3	30.62±5.15	4.37±4.00	2.95±0.07^{a}	–	5.39±2.50^{a}	5.37±1.92	23.58±2.77
	T7	26.37±6.52	5.25±4.27	3.75±1.98^{a}	–	8.75±3.58^{a}	6.25±1.98	20.39±3.29
	T10	32.62±4.14	5.37±3.81^{a}	4.25±1.67^{a}	–	9.25±3.33^{a}	5.38±2.26	25.63±4.57

Data are expressed as mean ± standard deviation.
n = 8 for each sampling point and each treatment; C – control group; AV – salmon fillets treated with aromatic vinegar; 2-PHE – 2-phenylethylamine; PUT – putrescine; CAD – cadaverine; HIS – histamine; TYR – tyramine; SPD – spermidine; SPM – spermine; T1 – 2 h; T3 – 3 days; T7 – 7 days; T10 – 10 days. Within each treatment, different superscript letters (x, y, z) indicate differences between storage periods ($p \leq 0.05$); within each storage period, different superscript letters (a, b) indicate differences between treatments ($p \leq 0.05$).

TABLE 3. Physical and chemical parameters (pH, colour and total volatile basic nitrogen values) of control and treated fillets throughout the storage experiment.

Attribute	Treatment	Storage time				SEM	P value		
		T1	T3	T7	T10		Tr	ST	Tr x ST
pH	C	6.13	6.08	6.17	6.10	0.0125	0.384	0.132	0.536
	AV	6.13	5.97	6.16	6.09				
L*	C	50.16	49.84	48.66	49.89	0.775	0.891	0.053	0.443
	AV	51.03	50.63	47.71	48.88				
a*	C	8.93^{w}	10.66^{x}	11.41^{x}	11.82^{x}	0.453	0.465	<0.001	0.156
	AV	10.33	10.87	11.40	11.16				
b*	C	14.02^{w}	16.10^{wx}	18.02^{x}	17.69^{x}	0.706	0.414	<0.001	0.265
	AV	15.71	17.18	17.57	17.01				
TVB-N (mg/100 g)	C	22.7^{w}	23.5^{w}	24.8^{x}	29.3	0.027	0.139	<0.001	0.110
	AV	21.9^{w}	23.8^{x}	24.8^{y}	28.7				

n = 10 samples for each treatment and sampling point; C – control group; AV – salmon fillets treated with aromatic vinegar; Tr – treatment; ST – Storage time; TVB-N – total volatile basic nitrogen; T1 – 2 h; T3 – 3 days; T7 – 7 days; T10 – 10 days. Within each storage period, different superscript letters (a, b) indicate differences between treatments ($p \leq 0.05$). Within each treatment, different superscript letters (w,x,y,z) indicate difference between storage times ($p \leq 0.05$).

not increase significantly during the experiment. Furthermore, the pH of the AV used was 6.2 and thus it was not able to alter the surface pH of the fish and induce protein denaturation.

The a_w values did not vary either for the treatment or during storage, ranging from 0.985 to 0.986 at T1 and from 0.983 to 0.982 at T3 for C and AV groups, respectively. The values were high enough to promote microbial growth and spoilage of the fillets.

Indeed, when selecting salmon fillets, surface colour and appearance are important indicators for consumer acceptance and product final price, since pigmentation intensity is considered a quality characteristic for the salmonids [Fidalgo *et al*., 2018; Lerfall *et al*., 2016; Wang *et al*., 2017a].

The initial values of L* (lightness), a* (redness), and b* (yellowness) were between 50.16 and 51.03, 8.93 and 10.33, and 14.02 and 15.71, respectively (Table 3). No significant differences were found in the L* values between the two groups throughout storage, and both groups showed a slight but not significant decrease in lightness. This finding was unexpected as generally the L* value increases during ice storage [Erikson & Misimi, 2008] but limited changes are reported in salmon fillets stored in MAP conditions [Gimenez *et al*., 2005].

The a* values increased during storage in the products but no difference was registered between the control and treated samples. These results are in accordance with those reported by Miraglia *et al*. [2016] in their study where *S. salar* raw fish was treated with a phenolic extract from olive vegetation water, and also by Fidalgo *et al*. [2018] for fresh Atlantic salmon during hyperbaric storage at room temperature. Moreover, the a* values were lower than those reported by Wang *et al*. [2017a] and by Fidalgo *et al*. [2018]. The b* value followed the same trend as reported for the a* value. The increasing presence of both a* and b* color components, reported also by other authors [Erikson & Misimi, 2008], could lead to a more brownish colour of the fillets, which could potentially affect their sensory quality. Despite no difference observed between the treated and untreated fillets, the AV seems to stabilize the a* and b* components, despite its pale–amber transparent colour not able to modify the original fillets colour.

However, the changes observed in salmon colour during storage may be caused mainly by microbial spoilage, enzymatic activity, oxidation processes, and different treatments applied to the samples, causing several structural changes to the muscle and variation of the colour parameters [Fidalgo *et al*., 2018].

TVB-N, which is constituted by trimethylamine (TMA-N), ammonia and other basic nitrogenous compounds, has been proposed as a quality indicator for spoilage in fresh and lightly preserved seafood [Dalgaard, 2000; Gram & Dalgaard, 2002; Pons-Sànchez-Cascado *et al*., 2006; Sallam, 2007] since it shows a close relationship with sensory score [Pons-Sànchez-Cascado *et al*., 2006]. High values of TVB-N are not desirable since they indicate the existence of nitrogenous compounds deriving from the degradation, operated by proteolytic bacteria, of molecules containing nitrogenous compounds, such as proteins and nucleic acids [Wang *et al*., 2017a]. In the current study, the initial TVB-N values (mg N/100 g of product) ranged from 21.9 in the treated samples to 22.7 in the control samples (Table 3). Increases in the TVB-N values were detected throughout the storage period of the experiment, reaching values of 29.3 and 28.3 mg N/100 g by day 10 in the control and treated samples, respectively. The samples analysed during the experiment were all below the maximum value of 35 mg N/100 g flesh stipulated by the EC guidelines for different species of raw fish [European Commission Decision 95/149/EC, 1995]. The initial content of TVB-N was higher than that reported by both

Zaragozà *et al*. [2014] and Sallam [2007] who found initial TVB-N values in control samples of Pacific salmon below 10 mg N/100 g; and values of 22.7 mg/100 g muscle were reached only on day 9. TVB-N increases are related to storage conditions, the activity of spoilage bacteria, and hygienic practices [Zaragozà *et al*., 2014], and TVB-N values are also affected by the species, season, harvesting area, age and sex of fish [Sallam, 2007]. The TVB-N content increased in all samples, which demonstrates their successive deterioration throughout the study.

CONCLUSIONS

The spoilage process in salmon fillets was monitored in this study *via* microbiological and physicochemical parameters in order to find some natural treatments that can influence microorganisms growth and their ability to produce BA, and therefore to obtain safe products. The hygienic level of the production was mostly high, as fillets showed a TVC always below 7 log CFU/g, even if a trend of increasing counts of *Enterobacteriaceae* and other spoiling bacteria was highlighted.

This preliminary study shows that the use of AV can really improve the overall hygienic quality of fresh salmon fillets stored in MAP, controlling the microbial populations in fish, such as *Pseudomonas* spp. and psychrotrophic loads. In particular, a significant effect was found on BAs production, with lower contents determined in the treated fillets for putrescine, cadaverine, tyramine, and histamine.

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