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Antimicrobial activity of natural compounds against *Listeria* spp. and their effects on sensory attributes in salmon (*Salmo salar*) and cod (*Gadus morhua*).

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Abstract

The application of natural preservatives on fresh fish has potential to extend shelf-life. In the present study, 8 essential oils (EOs) (lemon, lemongrass, lime, garlic, onion, oregano, thyme and rosemary) and 3 organic acids (OAs) (ascorbic, citric and lactic) were evaluated. The antimicrobial activity of these compounds was tested *in-vitro* against four confirmed *Listeria* spp. isolated from retail skin-packed salmon and cod. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were established for each compound. Then, a sensory evaluation was performed by a panel of 'expert assessors' on cooked fish treated with all of the OAs and any 4 EOs with a MIC <0.8%. A series of descriptors were assigned to characterize the combination of each compound with cooked salmon or cod.

The highest antimicrobial effect against all *Listeria* spp. was observed for lactic acid (0.31-2.5%), but treatment with this compound resulted in the development of organoleptically unacceptable changes in salmon or cod. The most acceptable OAs for salmon and cod were ascorbic acid (1.25%) and citric acid (0.63%) respectively, which were shown to enhance certain organoleptic characteristics.

The most effective EO against all *Listeria* strains evaluated was oregano oil (0.2%) and it was considered suitable as a treatment for salmon. In contrast, none of the EOs tested was organoleptically acceptable in combination with cod because of their strong odours and flavours that masked the fresh attributes associated with this fish.

1. Introduction

Cod and salmon are two of the most consumed species within the European Union (EU) with a consumption of 2.32 and 2.17 kg per capita/year, respectively. Moreover, 99% of cod consumed in the EU is wild while salmon is farmed according to the most recent data available (EUMOFA, 2017).

Fish is a highly perishable product subjected to rapid autolytic and microbiological changes which increased under inadequate handling and storage conditions (James, 1986). However, not all microorganisms contribute to a deterioration in fish quality with specific spoilage organisms (SSO) being mainly responsible for producing organoleptic changes that lead to consumer rejection (Gram & Huss, 1996). SSO usually out-compete pathogenic bacterial species, with spoilage occurring before pathogenic populations reach hazardous levels. Nevertheless, *Listeria monocytogenes* may be an exception, since even its presence in low numbers can represent a significant food hazard, particularly among at risk populations (pregnant, elderly and immunocompromised) (Skara, Rosnes, & Leadley, 2012). Moreover, in Europe, the number of confirmed cases of listeriosis in humans has increased considerably in the last 5 years. In certain ready-to-eat (RTE) foods, the proportion of *Listeria monocytogenes* positive samples at retail level was the highest in fish products (EFSA & ECDC, 2017). Therefore, controlling the growth of spoilage and pathogenic microorganism remains a significant challenge for the seafood industry.

The use of natural preservatives, to extend the shelf-life of fish, has been widely studied by many researchers in the last decades (Alfonzo et al., 2017; Karoui & Hassoun, 2017; Li et al., 2012; Ozogul et al., 2017). Fish muscle can be considered sterile immediately after slaughter (Horsley, 1973). Hence the initial bacterial contamination immediately *post-mortem* is located mainly on exterior surfaces and

72 decontamination strategies may be targeted there. Surface decontamination
73 methods can be divided into physical and chemical. Among chemical compounds,
74 organic acids (OAs) and essential oils (EOs) have shown potential as they can have
75 a bactericidal effect. Such compounds can cause alterations of some physiological
76 cell processes or disruption of membranes or other cellular components (Loretz,
77 Stephan, & Zweifel, 2010).

78 Although no theory fully explains the mode of action of OAs, the effect of an initial pH
79 drop can result in reductions in bacterial levels (Skara, Rosnes, & Leadley, 2012). It
80 has been suggested that this antimicrobial activity may be caused by two primary
81 mechanisms: (i) cytoplasmic acidification and (ii) accumulation of dissociated acid
82 anions to toxic levels (Taylor et al., 2012).

83 EOs are aromatic oily liquids extracted from plant materials which are the widely
84 used as natural antimicrobial compounds (Burt, 2004). Their effectiveness is
85 generally not immediate in terms of reducing initial microbial populations, rather they
86 act as bacteriostatic compounds which can inhibit growth and extend shelf-life
87 (Harpaz, Glatman, Drabkin, & Gelman, 2003). However, their mechanisms of action
88 are not completely understood, EOs are constituted by different compounds, so
89 attributing antimicrobial activity to each one is difficult (Bajpai, Baek, & Kang, 2012).
90 It has been suggested that their antimicrobial effect may be due to the phenolic
91 nature of EOs (Shapira & Mimran, 2007). Phenolic compounds can disrupt the cell
92 membrane negatively affecting certain functional properties of the cell and possibly
93 leading to leakage of contents (Bajpai, Baek, & Kang, 2012).

94 The aim of this investigation was to assess the *in-vitro* effect of several EOs and
95 OAs against 4 wild strains of *Listeria* spp. and on the sensory characteristics of

96 salmon and cod in order to determine which would be microbiologically effective and
97 organoleptically acceptable for use.

98

99 **2. Material and methods**

100 **2.1. Antimicrobial substances**

101 Lemongrass oil, East Indian (CAS 8007-02-1), Lemon oil (CAS 8008-56-8), Lime oil
102 (CAS 8008-26-2), Garlic oil (CAS 8000-78-0), Oregano oil (CAS 8007-11-2), Garlic
103 oil, Chinese (CAS 8000-78-0), Onion oil, Dutch (CAS 8000-72-0), Rosemary oil
104 (CAS 8000-25-7), Thyme oil (CAS 8007-46-3), Lactic acid (CAS 50-21-5), Citric acid
105 (CAS 77-92-9) and Ascorbic acid (CAS 50-81-7) were provided by Sigma-Aldrich
106 (Sigma-Aldrich Ireland Ltd., Wicklow).

107

108 **2.2. Bacterial strains**

109 Six different bacterial wild strains were isolated from retail skin-packed raw cod and
110 salmon samples. Salmon samples (25 g) were aseptically transferred to stomacher
111 bags (Stomacher ® 400 classic, Seward) containing 225 mL of maximum recovery
112 diluent (MRD, Oxoid) and were homogenized in a Stomacher (Lab-blender 400,
113 Seward) for 1 min. From each bag, 0.33 mL aliquots were spread in triplicate in
114 Chromogenic *Listeria* agar (supplements: Chromogenic *Listeria* Selective
115 Supplement [ISO] and Brilliance *Listeria* Differential supplement, Oxoid) and
116 incubated for 24 h at 37°C. Six different colonies were streaked onto new plates to
117 ensure the culture purity. Following incubation, a single colony of each isolate was
118 transferred to 5 ml of Tryptic soy broth (TSB) and incubated 24 h at 37°C. Then, 1 ml
119 was transferred to sterile Eppendorf tubes and centrifuged for 5 min at 10,000 rpm

120 (Eppendorf centrifuge, model 5417 R, Eppendorf AG 22331, Hamburg, Germany).
121 The TSB supernatant was discarded, and the pellets were resuspended in MRD and
122 centrifuged again, this process was repeated twice. Final pellets were resuspended
123 in 500 µl of lysis buffer (Fisher Scientific, New Hampshire, US) and sent for
124 sequencing by partial 16S rRNA gene analysis, to an external laboratory (Eurofins
125 Medigenomix GmbH, Ebersberg, Germany). The taxonomic identification was
126 performed with the Basic Local Alignment Search Tool (BLAST) from the US
127 National Centre for Biotechnology Information (NCBI) database
128 (https://blast.ncbi.nlm.nih.gov). Four of the isolates were confirmed as *Listeria* spp.
129 and were stored at -80°C on Protect ™ beads until required (Technical Services
130 Consultants Ltd, Lancashire, UK).

131

132 **2.3. Antimicrobial activity of EOs and OAs**

133 The minimum inhibitory concentration (MIC) and minimum bactericidal concentration
134 (MBC) for antimicrobial compound were determined by the microdilution method as
135 described by McDermott (2005). Briefly, serial two-fold dilutions of EOs and OAs
136 (from 0.8 to 0.00625 % and 5.0 to 0.08%, respectively) were prepared in sterile
137 distilled water. For EOs, emulsions in 0.5% Tween 80 (Sigma-Aldrich) were prepared
138 (Shojaee-Aliabadi, Hosseini, & Mirmoghtadaie, 2017). Then, both preparations were
139 immersed in an ultrasonication bath (Ultrawave Limited, Cardiff, United Kingdom) for
140 15 min to enhance the solution/emulsion formation (Ozogul et al., 2017). The pH of
141 all emulsions and solutions was also measured at the maximum concentrations
142 tested (0.8% for EOs and 5% for OAs) by using a digital pH-meter (Crison
143 Instruments, Barcelona, Spain).

The frozen stored bacterial isolates were resuscitated in Tryptic Soya Broth (TBS) at 37°C overnight. A loopful was then streaked onto the *Listeria* chromogenic agar and incubated as before to ensure the absence of contamination in the culture. After incubation, a single colony was transferred to a tube containing 10 ml of TSB which was incubated overnight at 37°C in a shaking incubator (160 rpm, Orbital shaker MaxQ^{TM}4000, ThermoFisher Scientific). The bacterial suspensions for the experiment were prepared by inoculating 1 ml of the overnight culture into 50ml of TSB containing sterile glass beads (to avoid bacterial clusters), and incubating at 37°C, as previously described, until the stationary phase (3-5 x 10^9 CFU/ml) was reached (Gayán, García-Gonzalo, Álvarez, & Condón, 2014). The cultures were then diluted in TSB and 100µl were added to each microplate well to yield a final concentration of 5 x 10^5 CFU/ml. The microtiter plates were incubated at 37 °C with gentle shaking at 150 rpm (Friedman, Henika, & Mandrellm, 2002) for 24 h and after incubation, growth was visually assessed. The MIC was defined as the lowest concentration of compound without visible growth (Clemente, Aznar, Silva, & Nerin, 2016; Lambert, Skandamis, Coote, & Nychas, 2001). For wells without visible growth, 100 µL was plated on brain heart infusion agar (BHI, Oxoid) and following incubation the number of colonies was counted. The MBC was defined as the lowest concentration of compound that resulted in a reduction of 99.9% of the initial bacterial inoculum (Clemente, Aznar, Salafranca, & Nerin, 2017; Duarte, Luis, Oleastro, & Domingues, 2016). Control samples were prepared in distilled water + 0.5% Tween 80 and all assays were performed at least in triplicate.

2.4. Fish sample preparation and treatment conditions

Raw salmon (*Salmo salar*) and cod (*Gadus morhua*) were purchased fresh in a local supermarket and were cut aseptically into fillet pieces of 50-60g with skin. Samples were immersed for 15 min in a sterile solution/emulsion of each OA or EO at their corresponding MICs as determined in the *in-vitro* studies described above. The OA solutions and EO emulsions were prepared in Erlenmeyer flasks (250 ml) as described above.

Samples were kept refrigerated at 5°C during treatments and were then aseptically drained using a plastic net for 15-20 min (Li et al., 2012). Then, ~10-15 g samples were cooked for 30-45 s in a microwave on medium power, in containers with lids suitable for cooking, immediately before serving them to the expert assessors.

2.5. Sensory analysis

Sensory analysis to evaluate salmon and cod in combination with all OAs and with four of the EOs tested was carried out by a panel of 'expert sensory assessors' as defined by the International Organization for Standardization (ISO, 2012). The panel consisted of 4 fish experts from the University of Zaragoza, with the necessary training and proven experience in sensory analysis. A brainstorming session was performed for each fish species in order to generate a number of sensory attributes (Greiff, Mathiassen, Misimi, Hersleth, & Aursand, 2015) representing appearance, odour, flavour and texture, based on terms used in the sensory assessment of fish (Seafish, 2010). The generated descriptors from the panel were shared at the end of each session and a number of these were selected by consensus (Chambers IV, 2018) to characterize each fish/compound combination.

192 **2.6. Instrumental colour analysis**

193 Instrumental colour analyses were carried out on four random locations of salmon
194 and cod surfaces treated with all OAs and 4 EOs, using an untreated sample as
195 control. A Chroma Meter (CR-400 Konica Minolta sensing, Inc. Japan) was used for
196 measuring the CIE L* (lightness), a* (redness) and b* (yellowness) parameters (CIE,
197 1976). Equipment was previously calibrated using a black and white standard as
198 recommended by the manufacturer.

199

200 **2.7. Statistical analysis**

201 Two-way ANOVA analyses with Bonferroni post-tests was used to compare each
202 treatment with control for colour parameters using GraphPad PRISM® 5.0 software
203 (GraphPad software, Inc., San Diego, CA, USA). Statistical significance was
204 assigned to comparisons with $p<0.05$.

205

206 **3. Results and Discussion**

207 Six suspect colonies isolated from salmon and cod on *Listeria* chromogenic agar
208 were sequenced by 16S rRNA gene analysis (Mardis, 2008). Following BLAST
209 analysis, the identified bacterial species with the confidence percentage of identity
210 values, as well as origin of each isolate, are presented in Table 1. The antimicrobial
211 activity of eight EOs and three OAs was then determined against four of the *Listeria*
212 spp. isolated (two *L. welshimeri* and two *L. monocytogenes*). The 'A' strains were
213 isolated from salmon, and 'B' strains from cod.

214 The MICs and MBCs obtained are shown in Table 2. The compounds were generally
215 active at the concentrations tested against all bacteria evaluated, however, wide

ranges in MIC and MBC values were observed between compounds. In general, EOs showed higher antimicrobial activity against all selected bacteria than OAs, with MIC values of 0.1% to > 0.8 % and 0.31 % to 2.50 % respectively.

Antimicrobial activity screening showed that the most effective EOs were lemongrass, garlic, oregano, and thyme as these substances showed higher activity when used at lower concentrations. For this reason, the range of concentrations for the EOs that showed values > 0.8 % was not extended. The values obtained for onion, rosemary, lemon and lime EOs (>0.8 %) are in agreement with the values reported by different authors (Aldana, Andrade-Ochoa, Aguilar, Contreras-Esquivel, & Nevarez-Moorillon, 2015; Barbosa et al., 2016; Fisher & Phillips, 2006; Santas, Almajano, & Carbó, 2010).

Oregano EO showed the strongest activity against *Listeria* (MIC 0.2 % for all *Listeria* spp. strains). This level of activity was the same for all 4 *Listeria* isolates tested, which is in contrast to the other EOs, such as lemongrass or garlic EO. The high bactericidal activity obtained for oregano EO was consistent with values obtained by other authors (Barbosa et al., 2016; Oussalah, Caillet, Saucier, & Lacroix, 2007; Santos et al., 2017). Thyme EO showed similar MIC values to oregano, but the MBC values were slightly higher which is also in agreement with other authors. Mith et al. (2014) observed similar MIC values for different thyme and oregano species against *L. monocytogenes* with higher MBC values reported for some of the strains. Iturriaga, Olabarrieta and Marañón (2012) also found similar MIC values for both EOs against *L. innocua* (ranging from 0.42 – 0.5%). In addition, Mazarrino et al., (2015) concluded that MIC concentrations of thyme and oregano exerted a similar bacteriostatic effect on *L. monocytogenes*, however the observed MIC values differed among strains.

Observed levels of activity for garlic and lemongrass EOs appeared to be strain dependent with *L. welshimeri* being more sensitive than *L. monocytogenes* to these active compounds. Lemongrass EO was more active than garlic EO, showing lower MIC and MBC values and similar findings have been previously reported in other studies (Kumral & Sahin, 2003; Raybaudi-Massilia, Mosqueda-Melgar, & Martin-Belloso, 2006).

In contrast, when organic acids were compared, similar MIC values were observed for ascorbic, citric and lactic acid for all *Listeria* strains tested (1.25, 0.63 and 0.31 %, respectively). Lactic acid was found to be the most active compound against *Listeria* spp. with a MIC value of 0.31% observed for all four strains which is in agreement with Huang, Lacroix, Daba and Simard (1993). However, MBC values were different between the strains tested, with a range of 0.63 to 2.50% observed. Citric acid was found to be the second most active OA, and similar to lactic acid, MIC values were the same for all 4 *Listeria* strains examined (0.63 %). Smaller differences in MBC values between strains were observed for citric acid, which is also in agreement with previous studies (Friedly et al., 2009). Ascorbic acid was the least effective against *Listeria* spp. and showed the same MIC values for all *Listeria* strains tested (1.25 %) and, in contrast to the other organic acids, MBC values were the same for all strains (2.50%). In this case, there was no difference in susceptibility between the *L. welshimeri* and *L. monocytogenes* isolates.

The pH values of solutions/emulsions for all compounds were measured at the maximum concentrations tested (0.8% - EOs and 5% - OAs) and are represented in Table 2. The pH of EO emulsions were found to be acidic with pH values ranging from of 5.04 (thyme oil) to 4.38 (lemongrass oil), which may contribute to the antimicrobial effect of EOs. It has been previously demonstrated that the

susceptibility of bacteria to EOs increases when the pH decreases (Burt, 2004). The hydrophobicity of an EO can increase at low pH, enabling dissolution in the lipids of the bacterial cell membrane (Juven, Kanner, Schved, & Weisslowicz, 1994). For OAs, pH values were 1.96, 1.98 and 3.04 for lactic, citric and ascorbic acid respectively and the main antimicrobial actions of weak organic acids is thought to be dependent on the low pH, and also the degree of dissociation of the acid (Lianou & Koutsoumanis, 2012).

Sensory evaluations were performed for salmon and cod treated with each active compound at the MIC concentration of the most resistant *Listeria* sp. evaluated. Sensory analyses were performed for all OAs and EOs that showed MICs lower than 0.8% as it was concluded that higher concentrations would result in fish being organoleptically unacceptable. During brainstorming sessions, the expert assessors generated and agreed on a number of descriptors for salmon and cod in combination with these 7 active compounds which are listed in Table 3. For salmon, the most suitable OA was ascorbic acid (1.25%) as it gave the product an enhanced salmon flavour (umami), while maintaining its normal texture and odour characteristics. Citric acid (0.63%) resulted in a lemon-like odour and flavour (citric), and negatively altered the texture making it dry and springy. Similarly, lactic acid (0.31%) also altered the fish texture, increasing the fibrosity and causing the development of off-odours and off-flavours, typical of spoiled fish (acid, rancid, blown oil). For EOs, garlic (0.4%), lemongrass (0.4%) and thyme (0.2%) oils were considered too strong in combination with salmon, hiding the organoleptic properties of salmon. However, oregano oil preserved the characteristics of fresh salmon without eclipsing its own flavour and odour.

For cod, the most suitable OA was the citric acid (0.63%) as it did not negatively alter the texture and improved some flavour and odour attributes (marine, aromatic, shellfish...). However, ascorbic (1.25%) and lactic (0.31%) neutralized the cod flavours, giving non-typical odours (vinegar-like, bready, fruity...). In general, the EOs were found to be not very compatible with cod as all of them masked any fish or seafood odour and flavour, and negatively affected the texture of the flesh (Table 3).

Negative sensory effects have been documented in seafood, poultry or vegetables treated with organic acids. The most frequently reported negative attributes are associated with acidic or vinegar-like odours and/or sour flavours (Chang & Fang, 2007; Kim & Marshall, 2000; Marshall & Kim, 1996). These attributes were also detected by the expert assessors panel for salmon and cod when treated with lactic acid. Other authors have also reported a reduction or neutralization of some characteristic flavours and odours of meat and meat products when treated with organic acids (Geomaras et al., 2005; Stivarius, Pohlman, Mcelyea, & Apple, 2002).

The use of EOs could have a negative impact on sensory attributes, even when used at low doses (Lv, Liang, Yuan, & Li, 2011; Sánchez-González, Vargas, González-Martínez, Chiralt, & Cháfer, 2011). Furthermore, their addition at high concentrations to fish products as a natural ingredient may cause allergic reactions as well as undesirable sensory changes (Hassoun & Çoban, 2017). Moreover, EOs can interact with some food components and if used at concentrations close to or exceeding 1% (v/w) could confer strong odours and flavours, leading to aftertastes (persistence) and bitter flavours, as occurred with most of the EOs tested in this study in both fish species (Hassoun & Çoban 2017; Mejlhom & Dalgaard, 2002). This is likely to adversely affect consumer acceptance (Ribeiro-Santos, Andrade, de Melo, & Sanches-Silva, 2017).

Instrumental colour analyses were also carried out on fish treated with the 7 compounds investigated in the sensory analysis. Colour parameters evaluated in raw salmon are shown in Figure 1. In general, all OA and EO treated samples were significantly different to their respective controls for each parameter (L*, a*, b*) evaluated. All treatments resulted in significantly higher L* values (lightness) and several also had lower levels on the redness (a*) and yellowness (b*) indices, which could explain the perceived bleached appearance of cooked salmon observed by the assessors panel. Bal'a and Marshall (1998) also observed a noticeable bleaching on catfish fillets after dipping in different organic acid solutions, with increasing L* values and decreasing a* values. This finding was also reported by Dehghani, Hosseini, Golmakani, Majdinasab and Esteghlal (2017) when rainbow trout fillets were treated with a coating containing certain essential oils. Colour changes have also been reported in meat and meat products treated with several organic acids and their salts (Anang, Rusul, Radu, Bakar, & Beuchat, 2006; Geomaras et al., 2005; Lu, Sebranek, Dickson, Mendoca, & Bailey, 2005).

Colour measurements of cod were carried out just after OA and EO treatments with results presented in Figure 2. As occurred in salmon, lightness values (L*) were significantly higher than controls, but no significant differences were found between control and treated samples in the a* index. However, cod is a white coloured fish *per se*, and increased L* and a* values may not be considered a negative effect. However, treatment of cod with lemongrass resulted in an increase in the yellow index (b*) when compared to respect to controls. This yellow colouration was also detected by the sensory panel assessors, and could be due to accumulation of pigmentation in the flesh as lemongrass is a dark yellow or dark amber colour (Skaria, Joy, Mathew, & Mathew, 2006).

4. Conclusions

The OA with the highest antimicrobial effect against *Listeria* spp. was lactic acid with MIC and MCB values ranging from 0.31 to 2.5%, depending on the strain. The essential oil most effective was oregano oil, where the MICs and MCBs of 0.2% were observed for 3 of the 4 *Listeria* spp. studied. Sensory evaluations of EOs with MIC values >0.8% were not carried out because they were considered too high and likely to be organoleptically unacceptable. The sensory evaluations carried out by the expert assessors highlighted a number of objective attributes for the combination of each OA or EO with salmon or cod. The OA considered most suitable for salmon from a sensory perspective was ascorbic acid and citric acid for cod. For EOs, none were considered suitable for cod due to their strong odours and flavours, which masked the original organoleptic properties of the fish. For salmon, oregano oil was found to be the most suitable EO that preserved the typical characteristics and despite being clearly perceptible, was pleasant and organoleptically acceptable.

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Figure 1

Colour measurements of raw salmon treated with organic acids (lactic, ascorbic and citric) and essential oils (thyme, garlic, lemongrass and oregano) and their respective controls. Each bar represents mean ± *SD*. (*) means significant differences ($p<0.05$) between each compound and control for each parameter (L*, a*, b*).

Figure 2

Colour measurements of raw cod treated with organic acids (lactic, ascorbic and citric) and essential oils (thyme, garlic, lemongrass and oregano and their respective controls. Each bar represents mean ± *SD*. (*) means significant differences ($p<0.05$) between each compound and control for each parameter (L*, a*, b*).

Figure 1

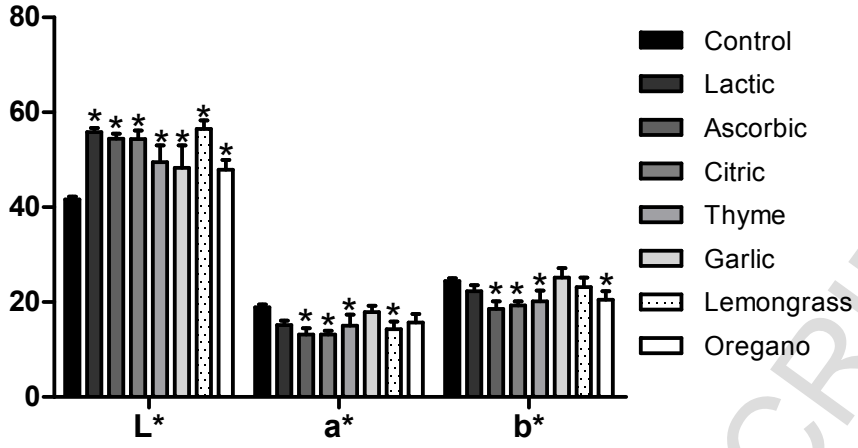
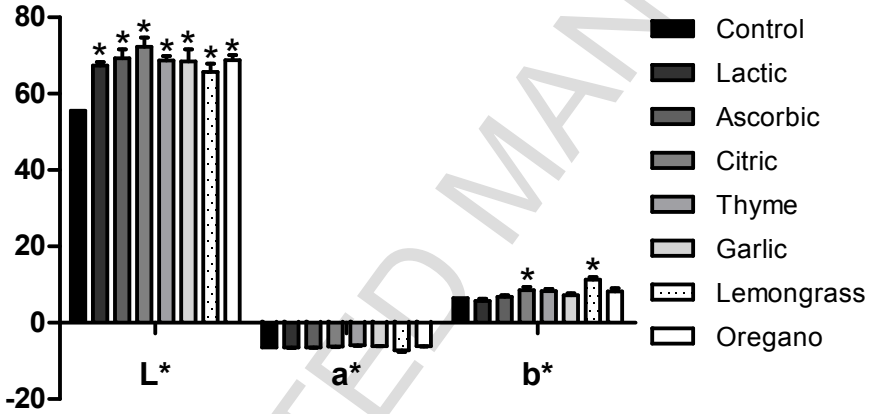


Figure 2



Highlights

- Lactic acid was the most effective organic acid against evaluated *Listeria* spp. *in-vitro*.
- Oregano showed the highest antimicrobial effect against evaluated *Listeria* spp.
- Ascorbic and citric acid proved to be the most suitable organic acids sensorially.
- None of the 4 essential oils tested in cod were organoleptically acceptable.
- Oregano oil was the most suitable essential oil from a sensory perspective for salmon.

Table 1. Bacterial species identification, isolated in this study from commercial fish, based on 16S rRNA gene analysis.

No.	Isolated from:	Ident. %	Species	Identification in the study
1	salmon skin-packed	99	*Listeria welshimeri*	*L. welshimeri*. A
2	cod skin-packed	89-90	*Serratia* spp.	*L.welshimeri*. B
3	cod skin-packed	99	*Listeria welshimeri*	
4	salmon skin-packed	99	*Listeria monocytogenes*	
5	salmon skin-packed	99	*Listeria monocytogenes*	*L. monocytogenes*. A
6	cod skin-packed	99	*Listeria monocytogenes*	*L. monocytogenes*. B

Table 2.

Antimicrobial susceptibility, expressed in term of minimal bactericidal concentration (MIC) (% (v/v)) and minimal bactericidal concentration (MBC) (% (v/v)) values of essential oil and organic acid against four *Listeria* spp. strains.

Compound		*L. welshimeri. A*		*L. welshimeri. B*		*L. monocytogenes. A*		*L. monocytogenes. B*	
	pH (*)	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Lemon oil	4.73 ± 0.01	> 0.8	> 0.8	> 0.8	> 0.8	> 0.8	> 0.8	> 0.8	> 0.8
Lemongrass oil	4.38 ± 0.02	0.1	0.4	0.1	0.4	0.4	0.4	0.2	0.4
Lime oil	4.73 ± 0.02	> 0.8	> 0.8	> 0.8	> 0.8	> 0.8	> 0.8	> 0.8	> 0.8
Garlic oil	4.94 ± 0.02	0.2	> 0.8	0.2	> 0.8	0.4	> 0.8	0.4	> 0.8
Onion oil	4.57 ± 0.01	> 0.8	> 0.8	> 0.8	> 0.8	> 0.8	> 0.8	> 0.8	> 0.8
Oregano oil	4.76 ± 0.01	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.4
Thyme oil	5.04 ± 0.01	0.2	0.4	0.2	0.4	0.2	0.4	0.2	0.4
Rosemary oil	4.62 ± 0.02	> 0.8	> 0.8	> 0.8	> 0.8	> 0.8	> 0.8	> 0.8	> 0.8
Ascorbic acid	3.04 ± 0.01	1.25	2.5	1.25	2.5	1.25	2.5	1.25	2.5
Citric acid	1.98 ± 0.02	0.63	1.25	0.63	2.5	0.63	2.5	0.63	2.5
Lactic acid	1.96 ± 0.03	0.31	0.63	0.31	1.25	0.31	2.5	0.31	0.63

(*) pH values were measured at the maximum concentration tested, 0.8% for essential oils and 5% for organic acids.

Table 3

Generated descriptors for salmon and cod in combination with organic acids or essential oils based on appearance, texture, flavour and odour attributes of fish.

Organic Acids		Salmon	Cod
Citric	*Appearance*	normal	normal
	Texture	dry, springy, very tough	firm, succulent
	Flavour	salmon, citric, a bit acid	seaweedy, shellfish, citric, a bit acid
	Odour	salmon, citric, aromatic	marine, shellfish, seaweedy, citric, aromatic
Ascorbic	*Appearance*	bleached	normal
	Texture	firm, succulent	watery, firm, a bit dry
	Flavour	salmon, umami	neutral
	Odour	salmon, marine, shellfish	bready, fruity, aromatic, mealy
Lactic	*Appearance*	normal	normal
	Texture	watery, fibrous	a bit firm, succulent, fibrous, crumbly
	Flavour	salmon, neutral, acid	neutral, bitter, acid
	Odour	rancid, blown oil, lactic acid	lactic acid, acetic acid
Essential oils			
Garlic	*Appearance*	bleached	normal
	Texture	watery, firm, succulent	firm, succulent
	Flavour	garlic, persistent	garlic, spicy, persistent
	Odour	garlic	garlic
Lemongrass	*Appearance*	yellowish	yellowish
	Texture	very firm, less juicy, dry	very firm, less juicy, a bit dry
	Flavour	bitter, persistent, flea repellent, lemon freshener	bitter, persistent, flea repellent, lemon freshener
	Odour	lemongrass, flea repellent, lemon freshener	lemongrass, flea repellent, lemon freshener
Thyme	*Appearance*	bleached	normal
	Texture	a bit dry, less juicy	a bit firm, succulent, fibrous, crumbly
	Flavour	thyme, spices	thyme, bitter, persistent
	Odour	thyme, spices	thyme, spices
Oregano	*Appearance*	normal	normal
	Texture	firm, dry	very dry
	Flavour	salmon, oregano, seasoned salmon	oregano, bitter
	Odour	salmon, oregano, seasoned salmon	oregano, bitter, camphor