Article

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Cold smoking of Lebranche mullet (*Mugil liza*): Physicochemical, sensory, and microbiological evaluation

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Abstract

The suitability of *Mugil liza* for cold smoking was evaluated and the effect of four different salting treatments on physicochemical, microbiological, and sensory characteristics was assessed. The addition of sucrose (3%) and acetic acid (0.5%) to the brine lead to fillet dehydration with lower NaCl penetration, combined with higher reduction of pH. Brining at refrigerated temperature ($5 \pm 1\,^{\circ}C$) instead of room temperature ($16 \pm 2\,^{\circ}C$) was more effective in preventing microbial growth. Loads of bacterial groups assessed in all smoked samples were below 100 CFU/g and the a_w, moisture, NaCl, and pH values achieved were within the typical stability range of smoked fish products. The obtained products were characterized by the *smoked aroma* and *salty taste*, differing in 7 of the 21 evaluated descriptors. The use of acetic acid in brine formulation showed an effect over color and texture descriptors. The affective test indicated the overall acceptability of products, being preferred the samples treated in the lowest NaCl concentration (5%).

Keywords

Antimicrobial agents, fish and fish products, omega-3 fatty acids, sensory analysis

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INTRODUCTION

Smoking is one of the oldest methods used for fish preservation. Originally, the process involved intense salting and smoking stages with the main objective of extending the shelf-life of the foods. It was attained by dehydration and reduction of a_w, combined with the antibacterial and preservative effects of chloride ions and smoke compounds. Nowadays, the smoking process has become a practice mainly performed to achieve food products with unique wood smoke flavors rather than for the preservation properties (Herrring and Smith, 2012).

Salting is the first main stage of the smoking process, in which fish acquires characteristics that impact the end product properties, like taste, texture, microbial development, shelf-life, and yield. During the smoking stage the product undergoes a dehydration process with the addition of smoke compounds that promotes further microbiological stabilization and hinders oxidative processes. This process can be classified as cold or hot smoking according to the temperature reached in the innermost part of the fish. Smoking modifies color, aroma, flavor, and texture, giving distinctive sensory attributes to the food (Herrring and Smith, 2012).

The rising global demand for processed seafood motivates the development of new products and a

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sustainable exploitation of resources by the use of alternative fish species. In this sense, smoking is an interesting option to provide diversified, high value-added products, since it is a technique of relative low complexity and low production cost. Moreover, this process may improve the overall fish product sensory acceptability and thus contributes to the inclusion of non-traditional fish species to the diet. Several studies dealing with the application of this technology to different fish species can be found in the literature (Adeyemi et al., 2013; Agustinelli and Yeannes, 2015; Gómez-Guillén et al., 2009; Guizani et al., 2014; Romotowska et al., 2016; Sérot et al., 2004). This research is focused on Lebranche mullet (*Mugil liza*), a species widely distributed throughout the coastal lines of the Atlantic Ocean and Caribbean Sea, stretching from the south of Florida, USA, to the argentine northern Patagonian waters (Menezes, 1983). In Argentina, *M. liza* is a non-traditional fish species exploited by a small scale artisanal fleet with low levels of captures, and commonly traded as fresh or frozen fillet at relatively low prices. Captures of *M. liza* are of major relevance in Brazil, with an average production of 11,930 tons/year from 2003 to 2017, representing volumes of captures 100 times greater than those reported for Argentine (IBAMA, 2007; MAGYP, 2010).

A few studies of cold smoked (Fernández et al., 1995) and hot smoked Lebranche mullet (Baptista, 2004) have shown promising results regarding the characteristics of the obtained product. However, more research is needed to generate technological knowledge in order to attain quality products consistent with current market quality demands.

The objective of this study is to characterize the cold smoking process of Lebranche mullet (*M. liza*), evaluating the effect of different salting conditions on the microbial populations, physicochemical parameters, and sensory properties of the end product in order to establish the technological conditions to obtain high quality cold smoked fillets.

MATERIALS AND METHODS

Raw material

Frozen skinless fillets (80) of Lebranche mullet (*M. liza*) were kindly provided by a local factory. Fish was caught during April/May 2015 in the Samborombón Bay, Argentina. Fillets were stored under freezing conditions (−23 °C) for two months until processing

Salting process

Fillets were salted in four different brine solutions: 5% w/v NaCl (S5), 7% w/v NaCl (S7), 10% w/v NaCl (S10), and 7% w/v NaCl + 3% w/v Sucrose + 0.5% v/v acetic acid (SSA). Sucrose was incorporated due to the osmo-dehydrating effect (Collignan et al., 2001; Collignan and Raoult-Wack, 1994). On the other hand, the acetic acid was added as an acidulant. These solutes represent the incorporation of new barriers to the microbial growth according to hurdle technology theory (Leistner, 2000). The brine–fish ratio was kept at 2:1. Two salting temperatures were used: room temperature (RoT), 16 ± 2 °C, and refrigerated temperature (ReT), 5 ± 1 °C. For each evaluated condition, samples ($n = 3$) were extracted after 3, 5, and 7 h of salting.

Smoking process

Fillets were salted and then cold smoked in an automatically controlled industrial smokehouse (Maurer-Atmos, Model ProfitLine PRR, Germany). Salting time was defined on the basis of previous salting experiments. Smoke was produced from a sawdust mixture of 50% "Virapitá" (*Peltophorum dubium*) and 50% "Virapere" (*Apuleia leiocarpa*). The process consisted of a three-phase program, with 15 min of drying, 210 min of smoking, and another 15 min of drying. Temperature was set at 20 ± 2 °C and the relative humidity at 50%. The internal temperature of the fillets rose from 6 to 17 °C at the end of the process. Smoked samples were vacuum packed and stored at 4 ± 1 °C for sensory analyses (up to 48 h) and at -18 ± 2 °C for physicochemical analyses (<15 days).

Physicochemical analyses

Proximate chemical composition of mullet fillets was determined according to AOAC methods: moisture (AOAC, 1990), ash (AOAC, 1993), lipid (AOAC, 1990), and protein (AOAC, 1993). NaCl content was determined by titration with a normalized $AgNO_3$ solution according to Mohr's method (Kirk et al., 1996). Acidity was determined by titration with NaOH solution (Pearson, 1970). pH measurements were carried out with a digital pH-meter Checker (HANNA, USA). Water activity (a_w) was assessed with a digital hygrometer Aqualab CX-2T (Decagon, USA). The thiobarbituric acid (TBA) index was determined by UV–vis spectroscopy (Shimadzu UV-1601 PC, Japan), at 532 nm for malondialdehyde and at 455 nm for other aldehydes, according to the procedure described by Tironi et al. (2007). For the determination of fatty acid composition, lipids were extracted with a chloroform/methanol mixture (Bligh and Dyer, 1959). Fatty acid profile was determined by gas chromatography, using KOH/methanol as transesterification agent, in a Shimadzu GC-17A chromatographer (Japan) equipped with a capillary column Omegawax 320 (Supelco, USA)

and FID detector. Injector and detector temperatures were held at 250 °C and oven temperature was set from 190 to 225 °C with a 1.5 °C/min ramp. Samples volumes were 1 µl and the carrier gas was nitrogen.

Determinations of physicochemical parameters were carried out in triplicate.

Color measurements

Instrumental color was determined with a portable colorimeter Lovibond SP60 (England), using the CIE *Lab* scale. Six measurements were performed on each side of five different fillets, per each sample. Hue and chroma values were calculated using the following equations (1) and (2), respectively

$$Hue = arctg\left(\frac{b^*}{a^*}\right) \quad (1)$$

$$Chroma = \sqrt{a^{*2} + b^{*2}} \quad (2)$$

Microbiological analyses

Samples taken at the end of salting and after smoking were immediately analyzed. Ten grams of fish muscle samples were homogenized in 90 ml of peptone water on a sterile container. Decimal dilutions were prepared for the following analyses: (i) total aerobic mesophilic bacteria (AMB) and (ii) total aerobic psychrotrophic bacteria (APB) on PCA incubated at 35 ± 1 °C for 48 h and at 4 ± 1 °C for 10 days, respectively; (iii) yeasts and molds (Y&M) on Yeast and Mold media at 25 ± 2 °C for 5 days; (iv) *Pseudomonas* spp on Cetrimide Agar Base at 35 ± 1 °C for 48 h; (v) *Staphylococcus* spp on Baird-Parker Agar Base at 35 ± 2 °C for 48 h; (vi) total coliforms on Violet Red Bile Agar at 35 ± 2 °C for 48 h; (vii) detection of *Escherichia coli* using Brilliant Green Bile Broth 2% in tubes at 35 ± 2 °C for 48 h; and (viii) lactic acid bacteria on MRS Agar Base incubated under anaerobic conditions at 35 ± 2 °C for 72 h. Culture media were from Britania®. Analyses were performed in duplicate.

Sensory evaluation

Quantitative descriptive sensory analysis was performed by 10 experienced judges in smoked fish products' sensory evaluation, trained according to Agustinelli and Yeannes (2015). The descriptive terms evaluated were *Aroma intensity, Smoked aroma, Salty aroma, Acid aroma, Sweet aroma, Oily aroma, Off aroma, Raw aroma, Color intensity, Brightness, Firmness, Fibrousness, Juiciness, Taste intensity, Smoked taste, Salty taste, Acid taste, Sweet taste, Oily aftertaste, Bitterness and Raw taste*. Attributes were quantified with a 10-point scale, where 0 = not detected and 10 = extremely strong. For the evaluation of taste, aroma, and texture attributes, sliced smoked fish samples were presented at RoT wrapped in foil to preserve the aroma. For appearance evaluation, the judges were given entire fillets to rate.

Consumer acceptability was assessed through an affective test carried out with a focus group consisting of 53 untrained panelists (55% men, 45% woman). Each panelist was asked to evaluate two samples and to rate them using a 7-point verbal scale, where 1 corresponds to "disliked extremely" and 7 to "liked extremely."

Sensory tests were carried out within the first 48 h of production, in individual cabins with controlled lighting and temperature.

Statistical analysis

Analyses of variance were carried out using the "R Project" Statistics Software, version 3.5 (R Development Core Team, 2018). Categorical qualitative data were analyzed by the Kruskal–Wallis test. Differences in pairs of mean values were evaluated by the Tukey test for a confidence level of 95%. Correlation analyses were carried out with Microsoft Excel (V16.0).

RESULTS AND DISCUSSION

Characterization of raw material

There is scarce literature about the characteristics of *M. liza* as raw material for food processes. The proximate composition given in g/100 g was 76.24 ± 1.14 of moisture, 5.15 ± 1.21 of lipids, 16.85 ± 1.05 of protein, and 1.23 ± 0.21 of ashes. These values are in accordance with those reported in literature for the species (Martínez, 2009; Valls et al., 2008). Table 1 summarizes fatty acid composition of *M. liza* fillets used in this investigation. Lipid fraction was dominated for PUFAs with the linoleic acid as the most abundant. The total of EPA + DHA accounts 19.67 g/100 g_{lipid}, which represents 1 g/100 g in fillet. These results indicate that the species is interesting from a nutritional point of view since consumption of long chain PUFAs has been widely related to beneficial effects in human health (Bagge et al., 2017).

Salting process

Changes on water and salt content through the salting process are shown in Figure 1. Water content increased on S5 and S7 after 3 and 5 h of treatment at both

Table 1. Fatty acid profile of raw Lebranche mullet fillets (*M. liza*) (g/100 g_{lipids})

SFA		PUFA		MUFA	
∑SFA	18.2	∑PUFA	45.9	∑MUFA	34.8
C 14:0	3.21	C 16:3	0.18	C 15:1	0.30
C 15:0	0.15	C 18:2 ω-6	20.42	C 16:1 ω-7	2.25
C 16:0	12.19	C 18:3 ω-6	0.06	C 17:1	0.27
C 18:0	2.35	C 18:3 ω-3	0.62	C 18:1 ω-7	1.30
		C 20:4 ω-6	0.27	C 18:1 ω-9	19.48
		C 20:4 ω-3	0.45	C 20:1 ω-11	0.29
		C 20:5 ω-3	3.41	C 20:1 ω-9	2.99
		C 21:5 ω-3	1.48	C 22:1 ω-11	5.42
		C 22:4 ω-6	0.19	C 22:1 ω-9	2.50
		C 22:5 ω-6	0.44		
		C 22:5 ω-3	0.43		
		C 26:6 ω-3	16.26		

MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; SFA: saturated fatty acids.

temperatures ($p < 0.05$), whereas S10 showed no significant change during the whole process (Figure 1(a)). On the other hand, SSA exhibited a continuous decline in the moisture during salting ($p < 0.05$), as shown in the inset graph of Figure 1(a). Regarding salt content, higher absorption rates were observed on samples treated in binary solutions of greater NaCl concentration (Figure 1(b)). After 3 h of salting, SSA showed lower salt levels than S7 and S10 ($p < 0.05$), which could be explained by the formation of a concentrated superficial layer of sucrose over the fillets that obstructs salt penetration (Collignan et al., 2001). The effect of temperature on salting was not significant ($p > 0.05$), even though fillets treated at RoT showed a slight tendency to reach lower moisture and higher salt contents than those at ReT.

The increase in moisture observed in S5 and S7 could be product of the swelling phenomenon described by Offer and Trinick (1983), also reported in other fish species (Gallart-Jornet et al., 2007; Czerner and Yeannes, 2013, among others). This observation is related to the relatively low salt levels in S5 and S7 fillets, corresponding to the range of maximum water holding capacity (Offer and Trinick, 1983).

The higher dehydration found in SSA is explained by the synergic effect of solutes used in the brine. It has been demonstrated that sucrose tends to stay on the surface of the fillet raising the solute concentration gradient between the fish and the solution, while salt diffuses into the muscle and induces water loss by osmotic effect at the cellular level (Collignan et al., 2001; Collignan and Raoult-Wack, 1994). Several authors have reported this effect on different fish species, e.g. Agustinelli et al. (2013) on carp and later on chub mackerel (Agustinelli et al., 2014). Likewise, acetic acid could also promote water loss owed to protein denaturation (Tribuzi et al., 2014).

The a_w showed a slight decreasing tendency with salting time, from 0.999 in raw fillets to values in the range 0.977–0.900 after 7 h. However, no statistically significant differences were found among samples nor with raw fillet ($p > 0.05$).

A noticeable effect of temperature was found in pH values of fillets treated in binary solutions, with no differences between brine compositions. pH was lowered from 6.40 ± 0.01 to 6.28 ± 0.02 in samples salted at RoT ($p < 0.05$) while it was constant in fillets processed at ReT. This effect could be linked to the slightly (yet not significant) higher salt content observed in fillets treated at RoT, since it is known that NaCl induces depolymerization of myofibrillar lattices that exposes polar or charged groups thus modifying muscle pH (Puolanne et al., 2001). SSA showed a progressive pH reduction during salting, reaching a final value of 5.09 ± 0.03 and, conversely, acidity increased from 0.22 ± 0.02 to 0.35 ± 0.004 $g_{acetic\ acid}/100$ g.

Results for microbiological counts determined during salting are shown in Table 2. Counts of AMB, APB, and Y&M were reduced on samples treated at ReT, reaching the minimum loads after 5 h. The effect of the use of low temperatures (ReT) was more pronounced on AMB counts, being 2–3 log cycles lower than those registered at RoT. Moreover, Y&M were reduced to <100 CFU/g in samples treated at ReT. On the other hand, treatments at RoT registered an increment on AMB and APB counts with salting time, which indicate that a prolonged treatment at RoTs could allow microbial development. Y&M remained constant in S5 and S7 at RoT and were reduced 2 log cycles in S10 and SAA.

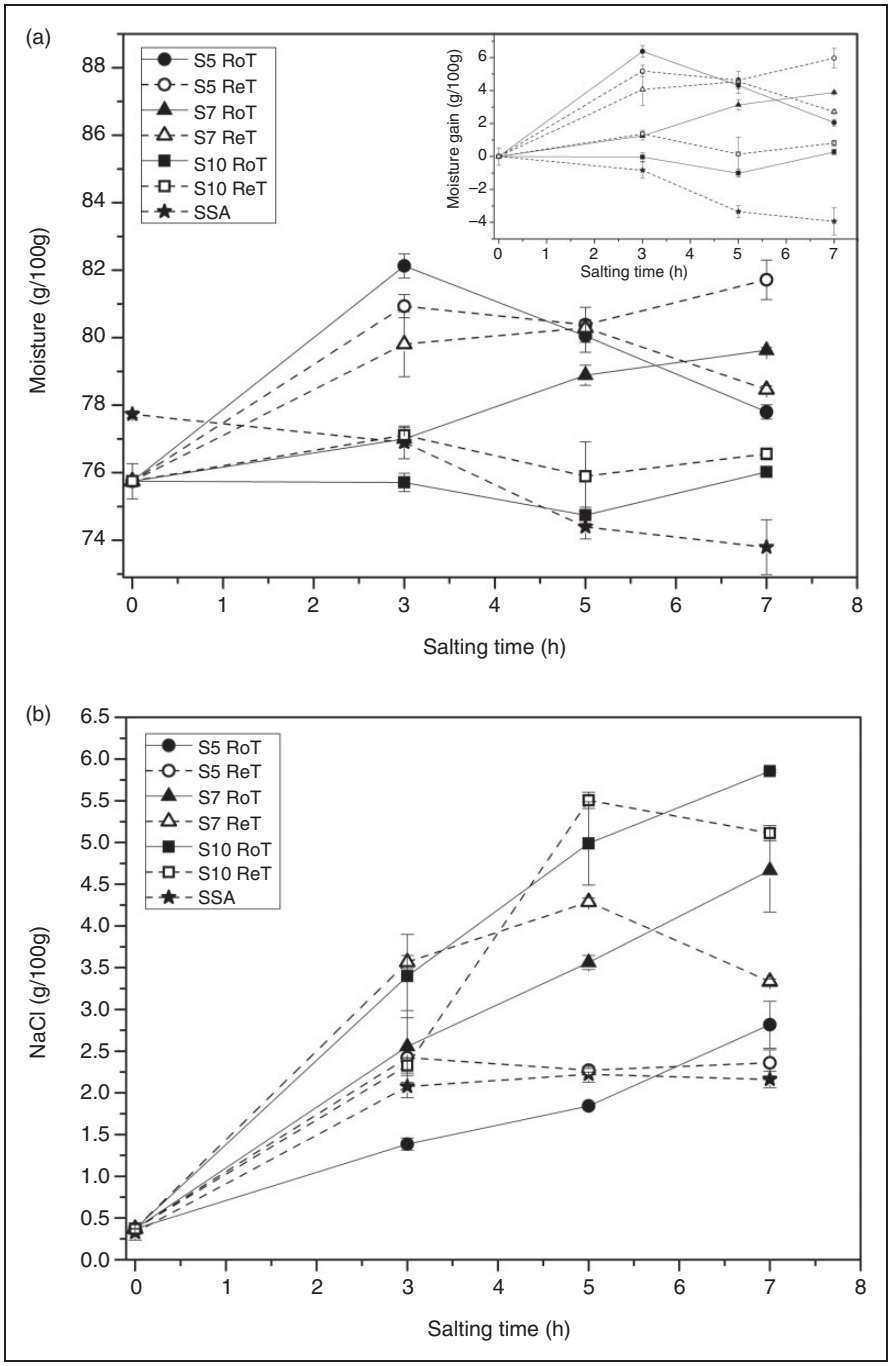

Figure 1. Changes in (a) moisture and (b) NaCl content during salting *M. liza* fillets, using brines of different compositions. ReT: refrigerated temperature; RoT: room temperature.

Staphylococcus spp loads were kept under 100 CFU/g in all samples and no presence of the assessed groups of pathogens was detected.

Based on the microbiological results, it was decided to carry out the salting stage under ReT for the smoking process. Also, brining time of 5 h was established and this would yield salt contents between 3 and 6% in all samples after smoking, values usually found in smoked fish products (Pedro and Nunes, 2019).

Smoking process

Changes in physicochemical parameters during the process are summarized in Table 3. Modifications of water, NaCl content, and pH of the different samples during the salting stage are in accordance with observations described in the "Salting process" section.

There is a noticeable decrease of water content in all samples after smoking, which causes solute concentration and therefore partially explains the increases

Table 2. Microbiological counts in Lebranche fillets (*M. liza*) during salting at different temperatures (mean ± SE)

Counts (log CFU/g)	Time (h)	S5		S7		S10		SSA
		RoT	ReT	RoT	ReT	RoT	ReT	ReT
AMB	0	3.37 ± 0.05Aa	3.30 ± 0.15Ba	3.37 ± 0.05Aa	3.30 ± 0.1Ba	3.37 ± 0.05Aa	3.30 ± 0.1Ba	2.57 ± 0.22B
	3	4.58 ± 0.01Ba	1.84 ± 0.09Ab	4.92 ± 0.03Ca	<2Ab	3.64 ± 0.1Ba	<2Aa	2.04 ± 0.15A
	5	4.2 ± 0.02BCa	<2Ab	3.92 ± 0.03Aa	<2Ab	3.83 ± 0.02Ba	<2Ab	2.41 ± 0.25B
	7	5.18 ± 0.01Ca	<2Ab	4.69 ± 0.05Ba	2.49 ± 0.01Ab	5.46 ± 0.06Ba	2.56 ± 0.01Ab	<2A
APB	0	5.59 ± 0.1Ba	5.59 ± 0.1Ba	5.59 ± 0.1Ba	5.59 ± 0.1Ba	5.59 ± 0.1Ba	5.59 ± 0.1Ba	4.61 ± 0.07B
	3	4.76 ± 0.05Aa	4.74 ± 0.12Aa	4.6 ± 0.04Aa	4.56 ± 0.12Aa	4.91 ± 0.18Aa	4.42 ± 0.09Ab	<2A
	5	4.93 ± 0.24Aa	4.65 ± 0.01Aa	5.11 ± 0.14Ba	4.33 ± 0.04Ab	4.63 ± 0.07Aa	4.39 ± 0.01Aa	<2A
	7	5.51 ± 0.11Ba	4.63 ± 0.12Ab	5.72 ± 0.06Ba	4.54 ± 0.15Ab	5.75 ± 0.18Ba	4.13 ± 0.01Ab	<2A
Y&M	0	4.09 ± 0.06Aa	4.09 ± 0.06Aa	4.09 ± 0.06A	4.09 ± 0.06A	4.09 ± 0.06A	4.09 ± 0.06A	4.09 ± 0.06A
	3	2.39 ± 0.55Ba	<2Ba	2.39 ± 0.12Ba	<2Ba	2.15 ± 0.21Ba	<2Ba	<2B
	5	3.39±0.49ABa	<2Bb	3.33 ± 0.41Ba	<2Bb	<2Ba	2.47 ± 0.67Bb	<2B
	7	4.1 ± 0.01Aa	<2Bb	3.39 ± 0.39Ba	<2Bb	2.54 ± 0.08Ba	<2Bb	<2B

AMB: total aerobic mesophilic bacteria; APB: total aerobic psychrotrophic bacteria; CFU: colony forming unit; ReT: refrigerated temperature; RoT: room temperature; Y&M: yeast and molds.

Different capital letters (A, B, C) in a column indicate differences ($p < 0.05$) between sampling times, for each microbial group. Different lowercase letters (a, b) indicate differences ($p < 0.05$) between different temperatures (RoT and ReT) for the same brine treatment.

Table 3. Physicochemical and microbiological parameters measured during *M. liza* smoking process (mean ± SE)

	Raw	S5		S7		S10		SSA	
		Salted	Smoked	Salted	Smoked	Salted	Smoked	Salted	Smoked
Moisture (g/100 g)	76.7 ± 1.1Y	82.1 ± 0.1ZCa	79.8 ± 0.7 ZDb	78.3 ± 0.1YBb	74.1 ± 0.1XCa	78.4 ± 0.7YAb	72.7 ± 0.1XBa	75.9 ± 0.4YAb	69.7 ± 0.5XAa
NaCl (g/100 g)	0.34 ± 0.02X	2.88 ± 0.1YBb	3.23 ± 0.03 YAa	2.71 ± 0.1YBb	4.02 ± 0.007YBa	5.03 ± 0.2YAb	6.42 ± 0.3YCa	2.37 ± 0.2YBb	3.83 ± 0.1YBa
A_w	0.999 ± 0.001Y	0.992 ± 0.001YBa	0.978 ± 0.002YBa	0.981 ± 0.009XABa	0.925 ± 0.005XAb	0.966 ± 0.004XAa	0.938 ± 0.011XAa	0.971 ± 0.003XAa	0.944 ± 0.019XAa
pH	6.39 ± 0.01Y	6.39 ± 0.02 YAa	5.85 ± 0.028XAb	6.23 ± 0.16YAa	5.68 ± 0.02XBb	6.39 ± 0.001YAa	5.63 ± 0.014XBb	5.42 ± 0.035XBa	5.2 ± 0.014XCb
Acidity ($g_{acetic\ acid}$/100 g)	0.22 ± 0.02Y	0.10 ± 0.01 XAa	0.16 ± 0.001XAb	0.18 ± 0.003 YBa	0.21 ± 0.003YBb	0.10 ± 0.01XAa	0.17 ± 0.005XABb	0.27 ± 0.005ZCa	0.39 ± 0.004ZCb
TBA_{455} (mg MDA/k)	0.402 ± 0.09Y	1.601 ± 0.14XAa	5.15 ± 0.18XAb	4.59 ± 0.08XBa	6.36 ± 0.88XABa	4.67 ± 0.02XBa	7.38 ± 0.52ZBb	4.53 ± 0.29XBa	5.87 ± 0.27XAb
TBA_{532} (mg aldehydes/k)	1.73 ± 0.20Y	3.84 ± 0.10ZAa	0.65 ± 0.02XAb	12.58 ± 1.17ZBa	1.07 ± 0.11XAb	12.89 ± 0.15ZBa	1.007 ± 0.06XAb	12.33 ± 0.46ZBa	0.72 ± 0.05XAb
AMB (log CFU/g)	2.52 ± 0.22Y	<2Z	<2Z	<2Z	<2Z	3.2 ± 2.6Y	<2Z	<2Z	<2Z
APB (log CFU/g)	4.60 ± 0.05X	<2Z	<2Z	<2Z	<2Z	<2Z	<2Z	<2Z	<2 Z
LAB (log CFU/g)	<2	<2	<2	<2	<2	<2	<2	<2	<2
Y&M (log CFU/g)	<2	<2	<2	<2	<2	<2	<2	<2	<2

AMB: total aerobic mesophilic bacteria; APB: total aerobic psychrotrophic bacteria; CFU: colony forming unit; LAB: lactic acid bacteria; MDA: malondialdehyde; TBA: thiobarbituric acid; Y&M: yeast and molds.

Different capital letters (X, Y, Z) in different columns indicate differences ($p < 0.05$) between each sample and the raw value. Different capital letters (A, B, C) indicate differences ($p < 0.05$) among samples in the same process stage. Different lowercase letters (a, b) within continuous columns (salted and smoked) indicate significant differences ($p < 0.05$).

observed in most of the physicochemical parameters assessed. For fillets treated in binary solutions, the higher NaCl content reached after salting led to higher loss of water during smoking. This result could be explained by the reduction of the water holding capacity once NaCl content in muscle exceeds approximately 1 M (considered as true solution in the liquid phase) (Offer and Trinick, 1983). Moreover, the higher loses of water observed in SSA can be attributed to the presence of acetic acid in the brine solution, which induces an additional degree of protein denaturation and thus favors water loss.

The differences in water and NaCl behavior between samples affected the smoking process yield, being 106.4% ($p < 0.05$), 104.5%, 103.8%, and 92.7% ($p < 0.05$) for S5, S7, S10, and SSA, respectively. These results indicate an increase on process yield as concentration of solutes in brine decreases, in accordance with results reported by Gallart-Jornet et al. (2007) for Atlantic salmon.

Water activity and pH/acidity are parameters indicative of the smoked fillets stability. As can be seen in Table 3, a_w showed a tendency to decrease in every process stage. S5 showed the lowest reduction of this parameter at the end of the process, which is linked to the higher water and lower salt content of these samples. The decreasing changes in pH and increasing acidity observed in the smoking phase could be explained by the deposition of aliphatic carboxylic acids from the smoke (e.g. formic and acetic acid) on the fillet surface. Other authors have reported similar results on smoked horse mackerel (Adeyemi et al., 2013) and smoked salmon (Loje, 2007). These parameters are expected to hinder the speed of spoilage reactions and hence to extend *M. liza* fillets' shelf-life. More studies are being carried out to evaluate the effectiveness of the different treatments on this matter.

Regarding rancidity, TBA_{532} index showed a fluctuating behavior, possibly related to the instability of malondialdehyde which is prone to react with other compounds (Aubourg, 1993). It is interesting that those samples with higher salt contents also showed, in general, higher levels of both TBA_{532} and TBA_{455}, which could indicate a positive correlation between lipid oxidation and salt concentration. It is well known that NaCl can promote oxidative reactions (Mariutti and Bragagnolo, 2017) as has been reported on other fish species (Guizani et al., 2014; Romotowska et al., 2016, among others). The TBA values reached are within the range reported by other authors for smoked fish products (Adeyemi et al., 2014; Yanar et al., 2006).

The salting–smoking processes used in this investigation were effective in reducing microbial populations (see Table 3). Microbial loads of the end product were lower than those found in raw material, because of the combination of the salt preservative effect and the antimicrobial effect of polyphenol compounds present in wood smoke (Sérot et al., 2004). Salting treatments had an inhibitory effect over the microbial groups studied, with the exception of AMB in S10. Moreover, counts of *Pseudomonas* spp, *Staphylococcus* spp, and total coliforms were all under 100 CFU/g and *Escherichia coli* was not detected in any sample.

Color changes. Lebranche mullet fillets exhibit different coloration on both sides, due to the anatomy of the fish. The inner side displays a homogeneous muscle appearance whereas the outer side usually is accompanied by rests of skin that gives it a different pigmentation. For this reason, color parameters were monitored on both sides of the fillet (Table 4).

As a result of brining, fillets showed a discoloration which could be evidenced by chroma values' reduction in all samples ($p < 0.05$) (with the exception of S10 outer side samples). Also, a shift in hue angle was detected from a predominant red-yellow to a yellow-green zone, especially on the inner side (Table 4), probably associated with the diffusion of myoglobin and traces of blood from the fish to the salting solution. The increase in L-value observed on the inner side of salted SSA fillets (Table 4) could be explained by the whitening effect of the acetic acid over muscle fibers. This phenomenon is related to the modification of the fillet's pH, that when close to the protein isoelectric point leads to a compaction of the myofibrillar lattice and thus more light is reflected by the muscle (Huff-Lonergan and Lonergan, 2005). The whitening effect continues being noticeable in smoked SSA samples.

After smoking, an increment of chroma values was observed, especially in the inner side of the fillets ($p < 0.05$), in comparison to salted samples (Table 4). Color changes in the smoking stage are related to the accumulation of colored phenolic compounds and to Maillard-type reactions that occur between carboxylic compounds of the smoke (mainly glycolaldehyde, glyoxal, and methylglyoxal) and amino acids of the protein muscle (Herrring and Smith, 2012).

It was noticeable the larger increase of chroma values observed in the inner side of smoked SSA fillets when compared to the other samples and to raw material. This higher development of color could be explained by the relatively low level of moisture in SSA that promotes the coagulation of superficial proteins during dehydration, forming a viscous external layer that enhances the adherence of the smoke coloring compounds (Herrring and Smith, 2012).

After smoking, the differences between the inner and the outer side color parameters were smaller than those observed in raw material, achieving homogenization of

Table 4. Chroma, hue, and *L* values of Lebranche mullet fillet (*M. liza*) through different stages of the process (mean ± SE)

		Salted				Smoked			
	Raw	S5	S7	S10	SSA	S5	S7	S10	SSA
Inner side									
Chroma	15.4 ± 2.2BCb	6.2 ± 2.4Ab	4.8 ± 1.3Ab	5.9 ± 1.6Ab	6.9 ± 1.3Ab	15.3 ± 2.7BCa	14.9 ± 2.8Ba	17.1 ± 2.4Ca	24.3 ± 4.2Da
Hue	82.4 ± 5.2Aa	119.2 ± 17.1Ca	131.3 ± 19.2Da	116.1 ± 19.2Ca	114 ± 12.8Ca	91.9 ± 5.4ABa	94.2 ± 5.9Ba	88 ± 3.8ABa	84.6 ± 3.7ABa
L	48.7 ± 5.8Aa	51.6 ± 4.2ACa	49.7 ± 4.4ABa	50.2 ± 4.1BCa	57.26 ± 3.5Da	48.7 ± 2.6Aa	49.8 ± 3.5ABa	49.16 ± ABa	54.3 ± 3.9CDa
Outer side									
Chroma	18.5 ± 3.7Da	12.9 ± 3.4Aba	11.9 ± 3.4Aa	15.7 ± 4.3BCDa	14.2 ± 3.6ACa	17.5 ± 2.7CDa	14.9 ± 3.1ACa	16.9 ± 2.5CDa	16.5 ± 5CDb
Hue	71.2 ± 4.9Ab	81.4 ± 9.8BCb	80.2 ± 8.4BCb	78.6 ± 6.9Bb	79.7 ± 6.2BCb	79.8 ± 5.6BCb	80.2 ± 8.4Ab	85.3 ± 4.6Ca	70.6 ± 5.5Ab
L	42.9 ± 5.6ABb	48.5 ± 4.9Cb	44.9 ± 3.5BCb	47.9 ± 4.2Cb	49.1 ± 4.6Cb	45.2 ± 3.5BCb	39.5 ± 3.6Ab	48.3 ± 3.5Ca	43.3 ± 4.6ABb

Values represent means of 30 replicates. Different upper case letters (A, B, C, D) indicate significant differences between columns ($p < 0.05$); Different lower case letters (a, b) indicate significant differences between inner and outer side for each sample ($p < 0.05$).

S5, S7, and S10 sample's chroma values and S10's *L* values, between both studied sides (Table 4).

Sensory analysis. According to the results of the descriptive qualitative analysis (DQA), the smoked products showed similar sensory profiles, differing only in 7 of the 21 sensory descriptors evaluated (Figure 2). All smoked Lebranche mullet fillets were rated with relatively high scores on *taste* and *aroma intensity* in comparison to the other descriptors, with *salty taste* and *smoke aroma* as the most outstanding characteristics of the product (Figure 2). These are related to the brining and smoking stages that lead to notable changes in the raw material (Herrring and Smith, 2012). The addition of sucrose to the brine in SSA had no effect over the sweet taste of the smoked products ($p > 0.05$) (Figure 2). Therefore, the use of sucrose under the conditions applied in this investigation allows taking advantage of its technological properties (e.g. greater dehydration with less salt diffusion) with no impact on the taste characteristics of the end product. Likewise, investigations on fish paste have shown that the combined use of acid and sugar exerts a reduction on the perception of sweet and acid tastes (Sánchez et al., 2010). Regarding appearance and texture, S5 was scored higher in *color intensity* and *brightness* than S7 and SSA, and lower in *firmness texture* than any of the other samples (Figure 2). These differences could be related to the higher moisture and lower NaCl levels of S5 samples.

Differences in taste descriptors were found between S10 and S5 and between SSA and S5 in the perception of *salty* and *acid*, respectively ($p < 0.05$) (Figure 2). In the first case, the samples correspond to the extremes of the range of NaCl content reached in smoked fillets (see Table 3). As to *acid taste*, it was notorious the difficulty of the judges to perceive it in SSA, since it was scored similar to the other samples that did not contain acetic acid (S10 and S7). This fact can be related to the suppressive effect that salt has over acid taste when both stimuli are present in foods (Breslin, 1996).

Scores of *acid taste* and *acid aroma* are directly correlated to the pH of the smoked samples, with $r = -0.98$ and $r = -0.91$, respectively. The better correlation coefficient of acid taste versus pH could be explained by the fact that the perceived intensity of *acid taste* is stronger than that of *acid aroma* when a judge is exposed to both stimuli (Garcia-Medina, 1981). Likewise, high correlations between water content and scores of *juiciness* ($r = 0.94$), *fibrousness* ($r = -0.92$), and *firmness* were found ($r = -0.72$). According to the DQA, it was evident the major role that acetic acid plays on firmness due to its softening/tenderization effect on the muscular fibers (Lück and Jager, 1997).

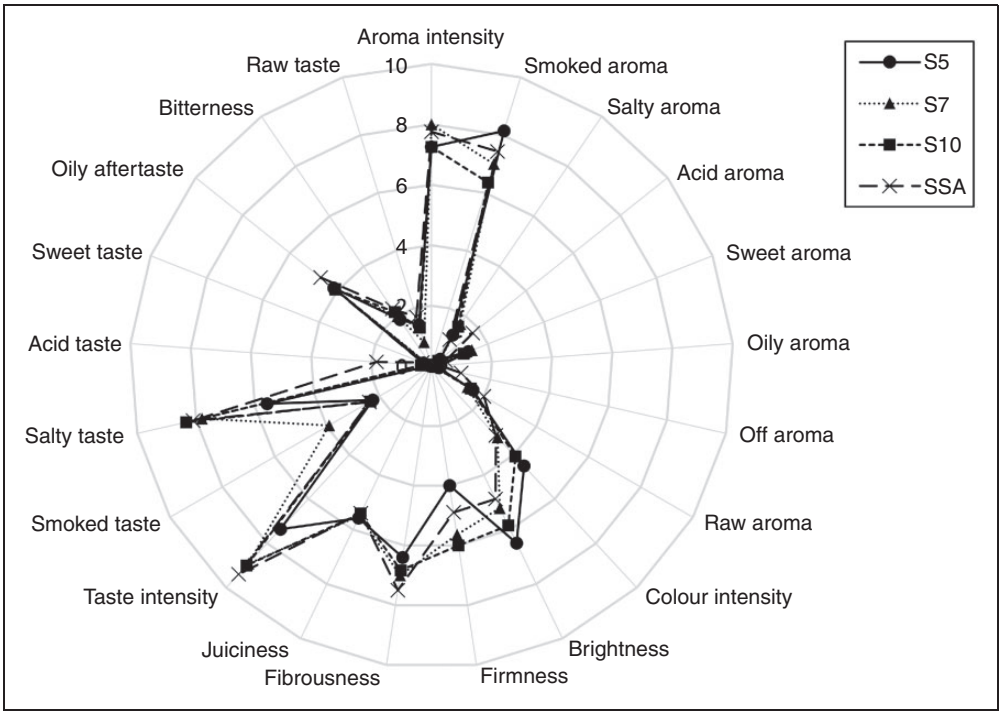

Figure 2. Sensory profile of smoked fillets of *M. liza*, treated with brines of different compositions.

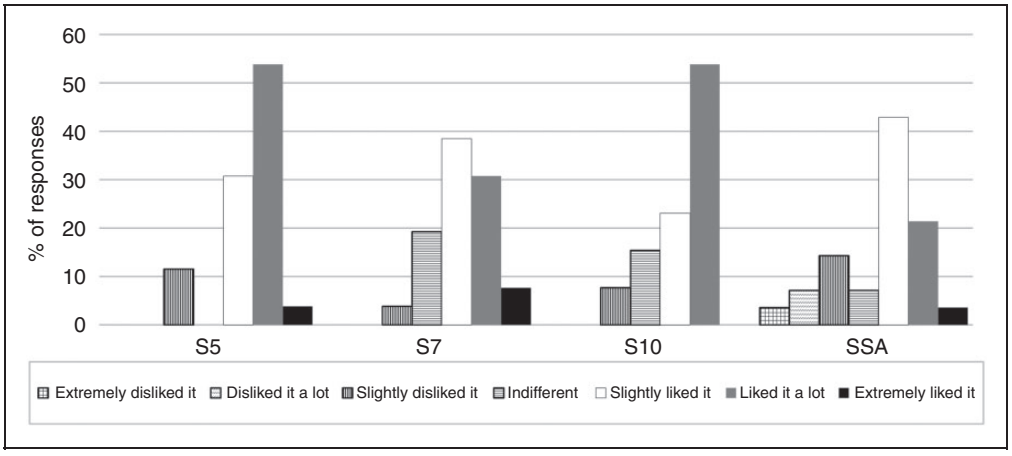

Figure 3. Frequency distribution of acceptability responses of smoked *M. liza* products.

The consumer acceptability analysis showed that all samples presented unimodal distribution, with modal values of 5 and 6 (Figure 3). This indicates that the smoked products developed were well accepted among consumers, being that the cumulative percentage of scores greater than 4 points were 88, 77, 77, and 68% for S5, S7, S10, and SSA, respectively. Data analysis over mean scores indicates that S5 fillets (5.39 ± 1.02) were mostly preferred over SSA samples (4.57 ± 1.45) ($p < 0.05$). As to the question of whether the consumer would buy or not buy the product, the survey indicates that there were sufficient affirmative (would buy) responses for samples S5 (81%) and S10 (77%) to consider them as statistically significant with a confidence level of 99.5 and 99%, respectively. It should be considered that the number of consumers that evaluated the acceptability of each sample (at least 26) is enough to consider the results obtained as representative of the population response at a laboratory scale (Pedrero and Pangborn, 1989). A survey with a large number of consumers would be necessary to validate these results marketwise.

CONCLUSIONS

The results of this research show that the employment of several conservation techniques at low intensity (salting, addition of sugars, acidification, smoking, dehydration), is effective to hinder microbial loads on smoked *M. liza* fillets and to obtain a fish product of mild sensory characteristics, in accordance with modern consumers' demands. Within the operational conditions evaluated, it was found that salting at ReT ($5 \pm 1\,^{\circ}C$) for a period of 5 h presented the best microbiological and physicochemical performance. Moreover, the addition of acetic acid and sucrose to the brine formulation led to the lowest pH levels and reduced NaCl content in smoked fillets.

Sensory analyses showed a high degree of similarity among samples when described by trained judges, but significant differences in consumers' acceptability analysis were found between fillets treated with NaCl 5% and those with NaCl 7% + sucrose 3% + acetic acid 0.5%. This could mean that slight modification on sensory attributes of this smoked product will have a great effect on consumers' response.

The products developed in this study could represent a major contribution to healthy diets of middle or lower income consumers, since *M. liza* is a species with a notable content of EPA and DHA with a healthy ratio of ω-6/ω-3 fatty acids, which would be more affordable than other similar smoked products.

These novel results represent the first scientific contribution on the processing of *M. liza*, strengthening the efforts to provide added value to maritime resources in order to diversify the fishing-based economy.

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