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Shelf-life evaluation of sliced cold-smoked rainbow trout (*Oncorhynchus mykiss*) under vacuum (PV) and modified atmosphere packaging (MAP)

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1 Shelf-life evaluation of sliced cold-smoked rainbow trout (*Oncorhynchus mykiss*)  
2 under vacuum (PV) and modified atmosphere packaging (MAP).

3  
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15

16 **Abstract**

17 Cold smoked trout (*Oncorhynchus mykiss*) is a traditional product from the San Daniele del Friuli  
18 area (Italy), usually packaged under vacuum, and its shelf life is 60 days. Aim of this study was to  
19 evaluate the influence of two different packaging systems (VP and MAP) on the microbial, chemical-  
20 physical and sensorial shelf life of sliced cold smoked rainbow trout, outlining the differences  
21 between the two methods. MAP packaging was found to better preserve the product, ensuring lower  
22 microbial loads throughout storage. VP packaged samples, in particular, exceeded the threshold limit  
23 of 6 log CFU/g after 60 days of storage. The TVB-N values, which were quite high at the beginning  
24 of the storage, increased over time in both treatments, reaching values close to the limit of 40 mg  
25 N/100 g after 45 days. Also TBARS values did not were up to 10 mmol/g in both VP and MAP.  
26 Based on the obtained data, the shelf life of 60 days was too long, particularly for the VP samples. A  
27 panel composed of 12 non-professional assessors established that a significant difference existed in  
28 the sensorial characteristic of the samples, thereby confirming the MAP packaged products had higher  
29 sensorial quality than the VP ones.

30

31 **Keywords:** cold smoked Rainbow trout, MAP, vacuum packaging, TVB-N, shelf life

32

33

## 34 **Introduction**

35 Smoked rainbow trout (*Oncorhynchus mykiss*) is a traditional product of Friuli, a region of  
36 northeastern Italy. Rainbow trout is farmed in that region, processed by cold-smoking and packaged  
37 after slicing under vacuum. The product is stored at  $4 \pm 2^\circ\text{C}$  and has a shelf life of approximately 60  
38 days. Being a cold-smoked fish, the product belongs to the category of mildly processed foods  
39 (Truelstrup Hansen and Huss, 1998). Fish is one of the most perishable food products: its initial  
40 microbial population reflects the microflora of the environment at the time of capture or harvest,  
41 and it is modified by the ability of various microorganisms (mainly bacteria) to multiply in the sub-  
42 environments provided by the skin/shell surface, gill areas, and intestinal content. The muscle tissue  
43 is normally sterile: microorganisms can be found on the skin and gills and in the intestinal tract  
44 (Baross and Liston, 1970; Shewan, 1977). The microbial loads depend on the water conditions and  
45 temperature: during storage, the microflora change, thus allowing the bacteria to tolerate the  
46 preservation conditions. The microbial ecology of cold-smoked fishery products has been  
47 intensively studied (Bernardi et al. 2009; Gonzàles-Rodrìguez *et al.*, 2002; Joffraud *et al.*, 2006;  
48 Lannelongue *et al.*, 1982; Leroi et al. 1998, 2001; Leroi, 2010; Lyhs *et al.*, 1998; Truelstrup Hansen  
49 and Huss, 1998). The initial bacterial load closely depends on the hygienic conditions of processing  
50 and production (Leroi *et al.*, 2001; Truelstrup Hansen and Huss, 1998), and it is represented by  
51 lactic acid bacteria (LAB), *Enterobacteriaceae*, *Shewanella putrefaciens*, *Aeromonas* spp.,  
52 *Pseudomonas* spp., *Photobacterium phosphoreum*, and *Brochothrix thermosphacta* (Cardinal *et al.*,  
53 2004; Civera *et al.*, 1995; Jorgensen and Huss, 1989; Leroi *et al.*, 2001, 2010; Lyhs *et al.*, 2007;  
54 Jaffrès *et al.*, 2008; Laurse, et al., 2005; Truelstrup Hansen *et al.*, 1996).

55

56 The refrigeration of cold smoked salmon and trout does not prevent the growth of abundant  
57 microflora, particularly LAB, which have a focal role in the microbial evolution occurring in the  
58 product. The shelf life of several cold smoked fish products is limited by the presence and growth  
59 of specific microflora, and although a certain correlation between these microflora and spoilage  
60 development has not yet been established, a level of microorganisms approximately 7-8 log CFU/g  
61 is considered to determine consumer rejection. These microflora could also be present at high loads  
62 without causing product spoilage; other factors are responsible for important microbial spoilage  
63 (Gram,1991; Gonzàles-Rodrìguez *et al* , 2002; Joffraud *et al* , 2001).

64 The dominant microflora in both vacuum-packed and MAP-packed smoked fish at the end of the  
65 retention period was often found to be LAB: the predominance of this group is not totally clear, but  
66 it seems that they are well suited to the particular pH, water phase salt (WPS) and salt concentration  
67 of smoked products (Bernardi *et al.*, 2009; Cardinal *et al.*, 2004; Laursen *et al.*, 2005; Leroi *et al.*,  
68 2000; Lyhs *et al.*, 2007; Samelis *et al.*, 1994). Also *Pseudomonas* spp. could be cause of spoilage in  
69 VP due to residual oxygen or to oxygen permeability of the protective film. *Pseudomonas* spp.  
70 could be the result of the initial contamination of the raw material. *Enterobacteriaceae* (Bernardi *et*  
71 *al.*, 2009; Gimenez and Dalgaard, 2004; Leroi, 2010) could be also present, they can growth either  
72 in VP or in MAP because they are anaerobic facultative, and consequently can produce acids and  
73 gas (CO<sub>2</sub>). Among them *Enterobacter* spp., *Serratia* spp., *Hafnia* spp. and *Proteus* spp. are the  
74 bacteria mainly present and are indicative of the level of hygiene in the processing (Bernardi *et al.*,  
75 2009). In the case of smoked salmon and related products, such as smoked trout, their role has not  
76 yet been clarified and they seem to have a minor role in the process of spoilage; many authors  
77 believe that there is no direct correlation between shelf-life and total counts of LAB (Leroi *et al.*,

78 2001), even though the LAB cause spoilage with the production of volatile sulphur compounds and  
79 amines (Bernardi *et al.*, 2011). The LAB, however, should not be considered harmful exclusively  
80 for the purpose of preservation, as they represent an important ally against potentially pathogenic  
81 microorganisms. Many studies (Gimenez and Dalgard, 2004; Joffraud *et al.*, 2006; Leroi, 2010;  
82 Leroi *et al.*, 1996) have reported that the presence of LAB, or their inoculation in smoked fishery  
83 products, results in growth inhibition of *Listeria monocytogenes*, *Clostridium botulinum* type E and  
84 *Salmonella* spp. This paper focuses on cold smoked trout fillets, a traditional product of the San  
85 Daniele del Friuli area that is usually packaged under vacuum (UV) after slicing.  
86 For the convenience of the consumer, because modified atmosphere packaging (MAP) allows for  
87 the easier separation of the slices, this study compared the shelf life of sliced cold-smoked rainbow  
88 trout packaged under vacuum to that of the same product with MAP packaging.

89

## 90 **Material and Methods**

### 91 *Samples*

92 Samples consisted of portions (200 g) of sliced cold-smoked rainbow trout packaged under vacuum  
93 (- 1.0 bar) or in MAP (- 0.3 bar; 70% N<sub>2</sub> and 30% CO<sub>2</sub>) , by Orved VM53 vacuum machine (Italy).  
94 The packaging consisted of Ecoterm VP 300 film (vacuum) and Multofog GA 170 (MAP;  
95 SUDPACK, Italy). The packages were stored at 4 ± 2 °C for 60 days and analysed after 0, 15, 30,  
96 45, and 60 days. Three packages at each time were used for microbiological and physico-chemical  
97 analyses. To produce the sliced cold-smoked rainbow trout used in the tests., two lots of raw  
98 material, were used. The trouts were farmed by Italian farm of San Daniele area (Friuli, Italy), they  
99 sized of 55 ± 5 cm , weighted 4.5 ± 5 kg, were put in ice water bath and beheaded, the post-mortem

100 period was about 24 h at 4 °C. Then they were smoked in smoking rooms with smoke temperature  
101 about 29 °C for 24 h.

#### 102 *Microbiological analysis*

103 Total aerobic mesophilic microbial counts (TMC) were evaluated on plate count agar (Oxoid, Italy)  
104 incubated at 30 °C for 48-72 h. LAB were counted onto De Man Rogosa Sharpe (MRS, Oxoid,  
105 Italy) after incubation under anaerobic conditions at 30 °C for 48 h; yeasts and moulds were  
106 counted onto malt agar (MA) (Oxoid, Italy) after incubation at 25 °C for 72-96 h. Total coliforms  
107 were counted on violet red bile lactose agar (VRBLA, Oxoid, Italy) and incubated at 37 °C for 24 h;  
108 coagulase-positive Staphylococci were enumerated on Baird-Parker agar medium (BP, Oxoid, Italy)  
109 supplemented with egg yolk tellurite emulsion (Oxoid, Italy) and incubated at 35 °C for 24-48 h;  
110 *Pseudomonas* spp. were counted on Pseudomonas agar base (Oxoid, Italy) supplemented with CFC  
111 (Oxoid, Italy) and incubated at 30 °C for 48 h; sulphite-reducing Clostridia were quantified on  
112 differential reinforced clostridial medium (DRCM, VWR, USA) after incubation at 37 °C for 24-48  
113 h in an anaerobic jar using an anaerobic kit (gas pack anaerobic system, BBL, Becton Dickinson,  
114 USA). *Listeria monocytogenes* and *Salmonella* spp. were investigated according to ISO methods  
115 11290/1 and 6579-1, respectively.

116

#### 117 *Physico-chemical analysis*

118 The pH value was measured using a pH meter (Basic 20, Crison Instruments, Spain), inserting the  
119 pH meter probe into 3 different points on each sample. Aw (water activity) was measured by Aqua  
120 Lab 4 TE (Decagon Devices, USA). The moisture content was measured by the A.O.A.C. (1990)  
121 method, NaCl and TVB-N (total volatile basic nitrogen) content were measured by the Pearson

122 method (1973). WPS was determined by the formula  $WPS = \% \text{ salt} / (\% \text{ salt} + \% \text{ moisture}) \times 100$ , as  
123 described by Huss *et al.*, (1997). Analyses were performed in triplicate per each sampling point. To  
124 evaluate the oxidation stability during storage, the Thiobarbituric acid–reactive substances  
125 (TBARS) were used in triplicate (Ke *et al.*, 1984).

126

### 127 *Sensorial analysis*

128 To evaluate the influence of UV and MAP packaging on the organoleptic characteristics of the  
129 sliced cold-smoked rainbow trout samples, a sensory analysis was performed using the triangle test  
130 methodology (ISO 4120:2004). Sensorial analyses were performed by 12 non-professional  
131 assessors. Four additional samples per each treatment were also evaluated after 60 days of storage  
132 at  $4 \pm 2$  °C. The non-professional assessors were presented with three products, two of which were  
133 identical. The assessors were asked to state which product they believed was the odd one out. The  
134 assessors who identified the different samples were asked to indicate their preference.

135

### 136 *Statistical analysis*

137 The values of the various parameters were compared by one-way analysis of variance. The averages  
138 were compared with Tukey's honest significance test using the Statistical Graphics software  
139 package.

140

## 141 **Results and Discussion**

142 The loads obtained from microbiological analyses of cold smoked trout fillets packaged under VP  
143 or in MAP are reported in Table 1. In the VP samples, the TMC was below the detection limit



144 (<100 CFU/g) at day 0, but a rapid increase after T15 was found, reaching at the end of the storage  
145 period a microbial count higher than the threshold limit of 6 log CFU/g often used in food industries  
146 to indicate the end of shelf life of fish products (Olafsdottir *et al.*, 2005). Conversely, in samples  
147 under MAP, the TMC was slightly higher at day 0, but the loads remained almost stable  
148 (approximately 2-3 log CFU/g) until day 45, at which point a rapid increase to a value of  $4.52 \pm 0.43$   
149 log CFU/g at day 60 was observed. It needs to be highlighted that a TMC of 7-8 log CFU/g, often  
150 associated with sensory rejection, was never reached. From T15 until the end of the storage period,  
151 the TMC values were significantly higher for the VP samples than for the MAP samples ( $p < 0.05$ ).  
152 Exactly the same trend was observed for the LAB counts. In fact, a rapid increase was observed  
153 during the storage period, with a jump of approximately 3 log from day 0 to day 15 and another  
154 jump of approximately 3 log from day 45 to day 60, when they reached a concentration of  $6.42 \pm$   
155  $0.67$  log CFU/g. Further, in this case, the LAB counts were significantly different between the  
156 treatments, thus confirming significantly higher values in the VP samples compared with the MAP  
157 samples ( $p < 0.05$ ), which were characterised by limited LAB growth throughout the period. Our  
158 values are similar or slightly lower than those reported in the literature for smoked vacuum-packed  
159 salmon: in those studies, the TMC values were approximately 7 to 8 log CFU/g, and LAB levels  
160 were between 5 and 6 log CFU/g (Bernardi *et al.*, 2009; Jaffrès *et al.*, 2008; Leroi *et al.*, 1998,  
161 2001; Leroi, 2010; Truelstrup Hansen *et al.*, 1996; Truelstrup Hansen and Huss, 1998). Dondero *et*  
162 *al.*, (2004) and Gonzàles-Rodrìguez *et al.*, (2002) obtained similar results in cold-smoked salmon  
163 and trout, even if the initial bacterial load, an index of the quality of the raw material and the  
164 hygiene of the process, was lower in our work than in these studies.

165 High-quality raw materials and good manufacturing practices were confirmed by the values of  
166 Coliforms and coagulase-positive Staphylococci (1 log CFU/g), which were always below the  
167 detection limit in both the VP and MAP samples during the monitored period, and by the absence of  
168 *Salmonella* spp. and *Listeria monocytogenes* in all samples.

169 The different types of packaging had an effect on *Pseudomonas* spp. growth (Table 1), particularly  
170 at the last two time points, where a rapid increase in the VP samples was observed. Conversely, in  
171 the MAP samples, *Pseudomonas* spp. were counted only at day 15 ( $2.00 \pm 0.07$ ), then they were no  
172 longer detected until the end of the storage period ( $< 100$  CFU/g). The contamination could either  
173 derive from the processing or originate from the raw materials.

174 According to data obtained from previous studies, their growth depends on the oxygen permeability  
175 of the vacuum packaging (Gonzàles-Rodríguez *et al.*, 2002; Leroi *et al.*, 1998, 2001). Sulphite-  
176 reducing clostridia were found only at day 60 in the VP samples. This result suggests that their  
177 presence cannot be connected to real growth but was likely sample-dependent.

178 Considering the physico-chemical analyses (Table 2), both pH and Aw showed a slight decrease  
179 over time without significant differences between the VP and MAP samples. The pH values started  
180 from 6.16 in both the VP and MAP products with no significant differences ( $p > 0.05$ ) during the  
181 storage period. Only at the end of the shelf life (T60), the pH decreased to 6.03 and 5.97 in the VP  
182 and MAP samples, respectively, and a significant difference was noted ( $p < 0.05$ ); this slight  
183 decrease could be due to the increase of Lactic Acid Bacteria that generally exert a buffer activity  
184 onto the food substrate. Leroi *et al.*. (1998) found quite stable pH values (from 6.04 to 6.25), as did  
185 Bernardi *et al.*, (2009), who reported constant values in the middle and at the end of the shelf life  
186 (6.12-6.11) in their study.

187 Similarly, the  $A_w$  values detected in the MAP samples were slightly lower than those measured for  
188 the VP samples, but the noticed differences were only significant at days 15 and 60 ( $p < 0.05$ ). The  
189 small observed  $A_w$  differences at the various sampling points probably depend on the samples'  
190 variability and are not related to real water loss. Moisture remained fairly constant over time. As far  
191 as the salt content and the WPS are concerned, the literature emphasises the importance of  
192 considering a WPS value of 3.5% as the minimum value able to control *Clostridium botulinum*, and  
193 in particular psychotropic type E, when combined with storage temperatures below 4.4 °C (Centre  
194 for Food Safety and Applied Nutrition, 2001). In this study, the values of salt and WPS varied  
195 during the storage period without displaying a specific trend, indicating that the observed  
196 differences depend only on the variability of the samples. The salt content was affected by the  
197 variability of the samples and of the salting procedure; for these reasons the decrease observed  
198 could not be considered a trend but a random heterogeneity of the samples.

199 Most of the studies met the guidelines suggested by the Centre for Food Safety and Applied  
200 Nutrition: Bernardi *et al.*, (2009) reported a mean WPS value in Italian smoked salmon products  
201 equal to 4.93%, whereas in French products the averages were lower but still approximately 4%  
202 (Cornu *et al.*, 2006; Hespe *et al.*, 2004). Moreover, differently from what found in the present study,  
203 Bernardi *et al.*, (2009) highlighted that WPS values lower than 3.5% were correlated with low  
204 bacterial counts. The mean TBARS values did not increased up to 10 nmol/g at the end of the  
205 storage (60 days) either in PV or in MAP samples. According to different authors (Ke *et al.*, 1984;  
206 Che Man and Ramadas, 1998) for quality evaluation, TBARS values of  $< 8$  nmol/g sample are  
207 considered not rancid, 9– 20 nmol/g slightly rancid but still acceptable and  $>21$  nmol/g rancid and  
208 unacceptable, consequently for the TBARS values, both the VP and MAP samples tested can be

209 considered still acceptable at 60 days, despite at 60 days 10 out of 12 assessors perceived a light  
210 rancidity and fluid loss in PV products and only 1 out of 12 perceived a light rancidity in MAP  
211 products. In PV products, the TBARS means at 60 days were significantly different compared to the  
212 ones at 0,15,30,45 days. In contrast no significant difference was present among TBARS means in  
213 MAP products at each tested times.

214 Considering the TVB-N results (Table 2), a moderate increase over time in both the treatments was  
215 observed. As early as day 45, the values were close to 40 mg N/100 g, which corresponds to the  
216 maximum value proposed by Cantoni *et al.*, (1993) for this parameter, with no significant  
217 differences observed between the treatments. Additionally, Bernardi *et al.*, (2009) found that at half  
218 the shelf life, the TVB-N values were close to the initial TVB-N limit (38.2 mg N/100 g), and at the  
219 end, they largely exceeded the suggested maximum value (49.8 mg N/100 g). The Chilean  
220 authorities (Sernapesca, 1996) established a limit of 30 mg N/100 g for cold smoked salmon, and  
221 this value was just present in our trial at day 0 for both the VP and MAP products. The observed  
222 TVB-N data were different from those reported by Leroi *et al.*, (1998), who found initial TVB-N  
223 values of approximately 15.5 mg N/100 g and final higher values equal to 52.8 mg N/100 g.  
224 A multiple compound quality index was proposed by Leroi *et al.*, (2001) to estimate the remaining  
225 shelf life time considering TVB-N and *Lactobacillus* spp. loads at storage temperatures < 5 °C.  
226 Applying this model and using the data of T30 for the VP and MAP products, the remaining shelf  
227 life was found to be 52 days and 55 days, respectively.

228 Considering the threshold limit of 6 log CFU/g (Olafsdottir *et al.*, 2005) for TMC and the TVB-N  
229 limit of 40 mg N/100 g (Cantoni *et al.*, 1993), the MAP products can be accepted until the end of  
230 their shelf life (60 days), but because the VP products exceeded the threshold limit of 6 log CFU/g,

231 this shelf life period is not adequate. MAP allowed to maintain higher structural and physical  
232 qualities than VP, wherein higher fluid loss occurred and less juiciness was noticed. However, the  
233 MAP composition must be carefully studied, as it can create unfavourable conditions for the  
234 microflora, hindering their ability to compete with pathogens, the replication of which may be  
235 facilitated to in this substrate, e.g., *C. botulinum* under anaerobic conditions (Huss, 1980),  
236 especially without a proper salt concentration.

237 The sensorial acceptability of both types of smoked rainbow trout, packaged VP or in MAP, was  
238 determined by the triangular test. The panel, which was composed of 12 non-professional assessors,  
239 established that a difference exists in the sensorial characteristic between the trout packaged in VP  
240 versus that in MAP, thereby confirming the physico-chemical and microbiological results. All the  
241 assessors indicated there were two distinct samples and identified that the MAP products had a  
242 higher sensorial quality than the VP (Table 3) also at the end of shelf-life.

243

#### 244 **Conclusion**

245 The sliced smoked rainbow trout samples, both vacuum and MAP packaged, were found to have  
246 good hygienic quality and to comply with the EC Regulation no. 2073/2005 as *Listeria*  
247 *monocytogenes*, *Salmonella* spp., coagulase-positive Staphylococci and Coliforms were never  
248 detected. MAP packaging preserved the product better compared with vacuum packaging, showing  
249 lower microbial loads throughout the storage period and at the end of shelf life.

250 However, some samples showed less than 3.5% WPS, the limit for the growth of psychrotrophic  
251 (non-proteolytic) *Clostridium botulinum*. As this bacterium could grow during the product's 60-day

252 commercial life, it is essential to optimise salting during the production process to ensure that an  
253 adequate concentration of NaCl is distributed homogeneously throughout the raw material.  
254 The deadline of 60 days appears to be too long, particularly for the VP samples, as confirmed by the  
255 sensory analysis. Consequently, a reduction of the "use-by" date could allow the manufacturer to  
256 guarantee the quality and safety of this product for its entire commercial life. **For PV products a**  
257 **shelf-life of 45 days is more reliable.**

258

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382 **Table 1: Microbiological analysis results (Log CFU/g) of the sliced cold smoked rainbow trout**  
 383 **packaged under vacuum (VP) and in Modified Atmosphere (MAP).**

Days	Total Mesophilic Count		<i>Pseudomonas</i> spp.		Lactic Acid Bacteria		Sulphite-reducing clostridia	
	VP	MAP	VP	MAP	VP	MAP	VP	MAP
<b>0</b>	< 100*	2.70±0.51	< 100*	< 100*	< 10*	1.30±0.41	< 10*	< 10*
<b>15</b>	4.29±0.50 <sub>a</sub>	2.24±0.34 <sub>b</sub>	< 100*	2.00±0.07	3.57±0.95 <sub>a</sub>	1.30±0.27 <sub>b</sub>	< 10*	< 10*
<b>30</b>	4.14±0.42 <sub>a</sub>	2.93±1.31 <sub>b</sub>	< 100*	< 100*	4.14±0.65 <sub>a</sub>	3.37±0.52 <sub>a</sub>	< 10*	< 10*
<b>45</b>	4.78±0.22 <sub>a</sub>	2.50±0.71 <sub>b</sub>	4.34±0.26	< 100*	3.53±1.27 <sub>a</sub>	1.97±1.68 <sub>a</sub>	< 10*	< 10*
<b>60</b>	6.21±0.29 <sub>a</sub>	4.52±0.43 <sub>b</sub>	6.66±0.01	< 100*	6.42±0.67 <sub>a</sub>	4.16±0.61 <sub>b</sub>	2.23±0.31	< 10*

Data represent the means ± standard deviations of the total samples; Mean with the same letters within a row (following the values), considering each single parameter, are not significantly differently ( $P < 0.05$ ). \*Value are expressed as CFU/g. Analyses were conducted in triplicate on three different samples per each sampling point.

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**Table 2: Results of physical-chemical analyses of the sliced, cold smoked rainbow trout packaged under vacuum (VP) and under Modified Atmosphere (MAP).**

		Days				
		0	15	30	45	60
% Water	VP	59.84±0.25 <sub>b</sub>	63.64±0.86 <sub>a</sub>	63.31±0.86 <sub>a</sub>	62.43±1.89 <sub>a</sub>	63.59±0.81 <sub>a</sub>
	MAP	61.22±0.10 <sub>a</sub>	63.37±3.04 <sub>a</sub>	61.77±4.27 <sub>a</sub>	64.01±2.10 <sub>a</sub>	62.93±0.61 <sub>a</sub>
% Salt	VP	3.11±0.03 <sub>b</sub>	2.18±0.59 <sub>a</sub>	2.69±0.14 <sub>a</sub>	2.78±0.16 <sub>a</sub>	2.59±0.23 <sub>a</sub>
	MAP	3.43±0.12 <sub>a</sub>	2.79±0.23 <sub>a</sub>	2.16±0.05 <sub>b</sub>	2.46±0.29 <sub>a</sub>	2.79±0.28 <sub>a</sub>
pH	VP	6.16±0.03 <sub>a</sub>	6.03±0.09 <sub>a</sub>	6.06±0.07 <sub>a</sub>	5.91±0.01 <sub>a</sub>	6.03±0.04 <sub>a</sub>
	MAP	6.16±0.04 <sub>a</sub>	5.98±0.03 <sub>a</sub>	6.16±0.04 <sub>a</sub>	5.96±0.06 <sub>a</sub>	5.97±0.01 <sub>b</sub>
Aw	VP	0.973±0.007 <sub>a</sub>	0.971±0.006 <sub>a</sub>	0.966±0.006 <sub>a</sub>	0.967±0.009 <sub>a</sub>	0.966±0.004 <sub>a</sub>
	MAP	0.963±0.008 <sub>a</sub>	0.963±0.001 <sub>b</sub>	0.963±0.004 <sub>a</sub>	0.968±0.002 <sub>a</sub>	0.958±0.002 <sub>b</sub>
% WPS	VP	4.94±0.15 <sub>b</sub>	3.32±0.91 <sub>a</sub>	4.07±0.16 <sub>a</sub>	4.27±0.21 <sub>a</sub>	3.85±0.28 <sub>a</sub>
	MAP	5.31±0.01 <sub>a</sub>	4.23±0.23 <sub>a</sub>	3.39±0.19 <sub>b</sub>	3.72±0.52 <sub>a</sub>	4.24±0.62 <sub>a</sub>
TVB-N mg N/100 g	VP	32.9±0.33 <sub>a</sub>	37.55±3.55 <sub>a</sub>	35.86±0.87 <sub>a</sub>	40.52±1.28 <sub>a</sub>	39.00±1.19 <sub>a</sub>
	MAP	32.61±1.39 <sub>a</sub>	34.95±1.38 <sub>a</sub>	36.19±0.23 <sub>a</sub>	39.20±1.45 <sub>a</sub>	39.35±0.64 <sub>a</sub>
TBARS nmol/g	VP	7.8 ± 1.2 <sub>a</sub>	8.1 ± 1.2 <sub>a</sub>	8.6 ± 0.5 <sub>a</sub>	9.2 ± 0.6 <sub>a</sub>	10.6 ± 0.3 <sub>b</sub>
	MAP	7.7 ± 1.3 <sub>a</sub>	7.9 ± 0.9 <sub>a</sub>	8.4 ± 1.8 <sub>a</sub>	9.3 ± 0.8 <sub>a</sub>	9.5 ± 0.6 <sub>a</sub>

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**Legend:** WPS: Water Salt Phase; TVB-N: Total volatile basic nitrogen; Data represent the means ± standard deviations of the total samples; Mean with the same letters within a column (following the values), considering each single parameter, are not significantly differently (P< 0.05). Analyses were conducted in triplicate on three different samples per each sampling point.

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**Table 3: Results of the sensorial analysis to determine**

405 the limit of acceptability by 12 assessors

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<b>Day</b>	<b>PV</b>		<b>MAP</b>	
	<b>A</b>	<b>NA</b>	<b>A</b>	<b>NA</b>
<b>0</b>	12		12	
<b>15</b>	12		12	
<b>30</b>	12		12	
<b>45</b>	12		12	
<b>60</b>	2	10*	11	1*

407 Legend: 12 assessors; A: acceptable; NA: Not acceptable

408 PV: Vacuum packaged; MAP: Modified Atmosphere

409 Packaging; 10\*: light rancidity/fluid loss/ less juiciness;

410 1\*: light rancidity.

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