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Additional Information

1 **Smoke-flavoured cod obtained by a new method using water vapour**
2 **permeable bags**

3

4 **Arantxa Rizo, Ana Fuentes*, Isabel Fernández-Segovia, José M. Barat**

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6 *Departamento de Tecnología de Alimentos. Universitat Politècnica de València,*

7 *Camino de Vera s/n, 46022 Valencia, Spain*

8

9

10

11

12

13

14

15 * Corresponding author at: Departamento de Tecnología de Alimentos, Universitat

16 Politècnica de València, C° de Vera s/n, 46022 Valencia, Spain Tel. +34 96 387 70 07;

17 ext. 73664 fax: +34 96 387 73 69.

18 E-mail address: anfuelo@upvnet.upv.es

19 **Abstract**

20

21 The objective of this study was to adapt and optimise a new smoking-salting process
22 developed for salmon to obtain smoke-flavoured cod. Fish was processed at 60%
23 relative humidity (RH)/5°C for 24 h using different salt doses. During process
24 optimisation, new conditions were studied (salt dose, RH, processing time). Smoke-
25 flavoured cod showed higher salt and moisture content than the salmon samples, which
26 required a higher salt concentration to reach similar a_w values. Process optimisation
27 allowed the exudate to evaporate when the process lasted 72 and 96 h. The samples
28 obtained with the 2% salt dose, 60% RH and 96 h gave the closest levels of moisture,
29 salt and a_w to commercial products. This new smoking-salting could substitute
30 traditional procedures as it minimises product handling and brine wastes, reduces
31 processing steps and can be applied to different fish types by adapting processing
32 parameters.

33

34 *Keywords:* salting, smoking, smoke-flavoured cod, water vapor permeable bags, liquid
35 smoke

36

37

38 **1. Introduction**

39 The smoking process has been employed since ancient times to preserve fish. The
40 traditional smoking process involves different stages, such as salting, drying and/or
41 smoking. Currently, there is growing interest in improving traditional smoking and
42 salting processes to minimise salt waste, reduce overall weight loss, improve hygienic
43 quality and ensure final product safety. Regarding this last aspect, smoked foods
44 generally involve health concerns, especially the possible presence of polycyclic
45 aromatic hydrocarbons. However, using smoke flavourings is generally considered a
46 less worrying health problem than the traditional smoking process since smoke
47 flavourings are produced from smoke subjected to fractionation and purification
48 processes (European Commission, 2003). For this reason, smoke-flavoured fish
49 production could be a good alternative to traditional smoked products.

50 In order to meet food industry and consumer requirements, Rizo et al. (2013) proposed
51 a new process to obtain smoke-flavoured salmon based on the combination of a
52 controlled salting process with smoked-flavoured salt and vacuum packaging. This
53 methodology was able to accelerate NaCl absorption and dehydration, reducing the total
54 processing time without affecting physico-chemical parameters compared with
55 traditional smoked salmon. In another study, Rizo et al. (2015a) proposed applying
56 water vapour permeable (WP) bags to improve the previously described smoking-
57 salting method. This methodology consists in simultaneous smoking-salting in WP bags
58 under established temperature and humidity conditions to control product dehydration.
59 This procedure allows smoke-flavoured salmon to be obtained with not only similar
60 physico-chemical traits and sensory acceptance to the smoked products obtained by
61 traditional methods, but also with good hygienic quality under cold storage (Rizo et al.,
62 2015a, b). The use of this new methodology could substitute traditional cold smoking of

63 fish because the physico-chemical properties, consumer acceptance and final product
64 safety are not affected, it minimises product handling and brine wastes, and reduces the
65 number of processing steps.

66 For the fish industry, applying the new smoking-salting method using WP bags to other
67 fish species could be of much interest. However, food processors need to optimise their
68 processes according to the characteristics of the raw material, and it is not always
69 possible to adapt previous procedures to new raw material characteristics or to final
70 product requirements. There are many factors that affect salting and drying processes,
71 such as species, muscle type, fish size, fillet thickness, weight, composition (lipid
72 content and distribution), physiological state, salting method, brine concentration,
73 salting step duration, fish-to-salt ratio, ambient temperature, freezing and thawing
74 (Ismail and Wootton 1992; Jittinandana et al., 2002; Wang et al., 2000). Lean fish
75 species like cod display a different behaviour during salting and drying to fatty species
76 like salmon (Cardinal et al., 2001; Gallart et al., 2007; Mørkøre et al., 2001).

77 The objectives of this study were to: (a) evaluate the feasibility of using a new smoking-
78 salting process developed for salmon to obtain smoke-flavoured cod; (b) optimise the
79 smoking-salting procedure to obtain smoke-flavoured cod.

80

81 **2. Material and Methods**

82 **2.1. Materials**

83 The fish employed as raw material were frozen cod (*Gadus morhua*) fillets of the 1.2-
84 1.4 kg commercial size (Alimentos Friorizados, S.A., Barcelona, Spain), which is the
85 commonest fish state used by the fish industry. Before processing, frozen cod fillets
86 (n=15) were thawed at 4°C for 24 h, trimmed to remove any bones and fins, and cut into

87 4-cm portions to obtain 5-6 portions per fillet (72 samples were obtained). The average
88 weight and thickness of fish portions were 136 ± 23 g and 3 cm, respectively.

89 The sodium chloride used for the smoking-salting process was supplied by Panreac
90 Química, S.A (Barcelona, Spain), and the natural liquid smoke HARDWOOD AFS 10
91 by Amcan ingredient Ltd. (Le Chesnay, France). Water vapour permeable bags were
92 supplied by TUB-EX ApS (Taars, Denmark) (size: $200\times 300\times 0.04$ mm; water vapour
93 transmission rate: $5000\text{g}/50\mu\text{m}^2/24\text{h}$ ($38^\circ\text{C}/50\%$ RH)). To determine the target physico-
94 chemical parameters for the new product, two different commercial smoked cod brands
95 were used. From each brand, two different batches were analysed. These products were
96 purchased from a local market in the city of Valencia (east Spain) and had been
97 processed by traditional cold-smoking techniques: dry salting, followed by a smoking
98 step in a smoking chamber.

99

100 **2.2. Experimental design**

101 *2.2.1. Phase I. Studying the feasibility of using a new smoking-salting process:*
102 *comparison between salmon and cod*

103 Cod portions were subjected to a simultaneous smoking-salting process (Fig. 1)
104 following the method developed by Rizo et al. (2015a) to obtain smoke-flavoured fish.
105 Fish samples were smoked by spraying the fillet surface with diluted liquid smoke (60
106 mL/100 mL solution) for 30 s. After applying liquid smoke, salting was carried out by
107 dosing a previously established amount of NaCl on the fillet surface. Three salt dose
108 concentrations were considered: 4, 6, and 8 g salt/100 g fresh fish. The amount of salt
109 added to each sample was calculated from the initial fish portion weight and the initial
110 water weight fraction (x^w) according to the procedure by Fuentes et al. (2008). Samples
111 were introduced into WP bags and were vacuum-packaged with a vacuum packaging

112 machine (Tecnotrip EV-25-CD, Barcelona, Spain). It should be noted that vacuum
113 packaging was used merely to ensure good initial contact between fish and the WP bag.
114 Then portions were processed in a drying chamber (Binder mod. KBF Tuttlingen,
115 Germany) at 60% RH for 24 h at 5°C. At the end of the processing time, samples were
116 removed from the bags and the exudate formed during the process was weighed. Fish
117 samples were introduced into saturated brine under constant stirring for 30 s to remove
118 any traces of salt attached to the surface. Finally, they were dried with absorbent paper,
119 weighed and left at 4°C for 24 h to ensure homogeneous salt distribution on the pieces.
120 Analyses of moisture, lipid content, NaCl content and a_w were carried out on the fresh
121 fish and the final product. Values considered as references were obtained from the
122 commercial smoked cod analysed (moisture, salt content and a_w).

123 **Fig. 1**

124

125 *2.2.2. Phase II. Optimisation of the smoking-salting process for cod*

126 Cod portions were submitted to the smoking-salting process described in Phase I with
127 some modifications (Fig. 2). In this study phase, the effects of different salt doses, RH
128 in the drying chamber and processing time on the physico-chemical properties of the
129 final product were studied. Processing variables were established after considering the
130 results obtained in Phase I and the physico-chemical characteristics of the target
131 product. Three salt dose concentrations (2, 3, and 4 g salt/100 g fresh cod), two levels of
132 RH in the drying chamber (60% and 70% RH), and three processing times (48, 72, and
133 96 h) were studied. The smoking-salting process was carried out at 10°C.

134 In both phases, three samples were used per condition (n=3) and the physico-chemical
135 analyses (moisture content, NaCl content and a_w) were run on each sample in triplicate.

136

Fig. 2

137

138 **2.3. Analytical determinations**

139 Moisture content (x^w) was determined by oven drying until a constant weight was
 140 reached at 105°C (AOAC, 1997). The lipid content (x^f) of the samples was determined
 141 by the AOAC method (AOAC, 1997). Sodium chloride content (x^{NaCl}) was established
 142 by the procedure described by Fuentes et al. (2010b), but using an automatic Sherwood
 143 Chloride Analyzer Model 926 (Sherwood Scientific Ltd., Cambridge, UK). Water
 144 activity (a_w) was measured in minced samples with an Aqualab dew point hygrometer,
 145 model 4TE (Decagon Devices, Inc., Washington, USA).

146 The total sodium chloride concentrations on a dry basis (X^{NaCl}), on a dry fat-free basis
 147 ($X_{\text{ff}}^{\text{NaCl}}$) and in the liquid phase (z^{NaCl}) were estimated by Eqs. (1)-(3).

$$148 \quad X^{\text{NaCl}} = \left(\frac{x^{\text{NaCl}}}{1-x^w} \right) \quad (1)$$

$$149 \quad X_{\text{ff}}^{\text{NaCl}} = \left(\frac{x^{\text{NaCl}}}{1-x^w-x^f} \right) \quad (2)$$

$$150 \quad z^{\text{NaCl}} = \left(\frac{x^{\text{NaCl}}}{x^w+x^{\text{NaCl}}} \right) \quad (3)$$

151

152 The total, water and sodium chloride weight changes (ΔM_t^o , ΔM_t^w and ΔM_t^{NaCl}
 153 respectively) of the fish samples were calculated by Eqs. (4)-(6).

$$154 \quad \Delta M_t^o = \left(\frac{M_t^o - M_0^o}{M_0^o} \right) \quad (4)$$

$$155 \quad \Delta M_t^w = \left(\frac{M_t^o \cdot x_t^w - M_0^o \cdot x_0^w}{M_0^o} \right) \quad (5)$$

156
$$\Delta M_t^{\text{NaCl}} = \left(\frac{M_t^0 \cdot x_t^{\text{NaCl}} - M_0^0 \cdot x_0^{\text{NaCl}}}{M_0^0} \right) \quad (6)$$

157 **2.4. Statistical analysis**

158 A multifactor ANOVA was conducted in Phase I for each physico-chemical parameter
159 to determine whether there were significant differences between salt dose, fish species
160 and their interactions. Likewise in Phase II, a multifactor ANOVA was performed for
161 each physico-chemical parameter to evaluate the effect of salt dose, RH during
162 smoking-salting, the processing time, and their interactions. The least significance
163 procedure (LSD) was used to test for the differences between averages at the 5% level
164 of significance. Data were statistically processed with Statgraphics Centurion XVI
165 (Manugistics Inc., Rockville, MD, USA).

166

167 **3. Results and Discussion**

168 *3.1 Characterisation of raw material and commercial smoked fish*

169 The physico-chemical parameters of the raw material and the commercial smoked cod
170 (brands A and B) are shown in Table 1. The moisture, salt content and a_w values in the
171 commercial samples were used to establish reference values.

172

Table 1

173

174 The values obtained in the physico-chemical characterisation of the raw material were
175 similar to those reported by other authors for fresh cod (Andrés et al., 2005; Gallart-
176 Jornet et al., 2007). In our study, the moisture and the lipid content of raw material was
177 83.55% and 0.16%, respectively. Compared with the other fish species used
178 traditionally for smoking, such as salmon, herring or sardine, cod has a very low fat
179 content, and therefore high moisture content. In salting processes, salt uptake depends
180 on many factors: the salting method, salt dose, freezing and thawing, and on intrinsic

181 fish factors like fish species, muscle thickness, postmortem state and composition
182 (Fuentes et al., 2008; Gallart et al., 2007; Jittinandana et al., 2002; Rørå et al., 1998;
183 Wang et al., 1998). For this reason, raw material characterisation prior to adjusting
184 smoking-salting conditions is essential (Barat et al., 2006).

185 The physico-chemical parameters of the commercial smoked cod fell within the range
186 of those reported by other authors (Karásková et al., 2011; Fuentes et al., 2010a).
187 Significant differences were found between the two brands analysed (ANOVA data not
188 shown). The largest difference was observed in salt content since the sodium chloride
189 content in brand A was twice as high as in brand B. Brand A also gave lower moisture
190 and a_w values compared to brand B, which could be attributed to greater intensity in
191 salting and/or smoking. Some studies have found wide variability for such products in
192 terms of their moisture content, salt and a_w , which could have implications for food
193 safety (Cornu et al., 2006; Espe et al., 2004; Fuentes et al., 2010a). These differences
194 directly influence sensory attributes and could determine shelf life, which can range
195 between 1 and 8 weeks as reported in other studies of smoked salmon (Cardinal et al.,
196 2004; Jørgensen et al., 2000; Leroi et al., 2001).

197 Given the wide variability found between brands, establishing single reference values
198 for moisture, salt and a_w is difficult. According to the Codex standard for smoked fish,
199 smoke-flavoured fish and smoked-dried fish (Codex, 2013), 5% aqueous phase salt
200 ($z^{\text{NaCl}} = 0.05$) would be required for smoke-flavoured fish in which smoke flavour is
201 provided by artificial flavour blends to provide complete protection against *Clostridium*
202 *botulinum* at temperatures between 3°C and 10°C. To fulfil this standard, the target
203 value selected for sodium chloride content in this study was ($x^{\text{NaCl}}=0.04$), which
204 corresponds to $z^{\text{NaCl}} = 0.05$ according to a moisture value of 74.2% (the average
205 moisture of both brands).

206 3.2. Phase I. Studying the feasibility of using a new smoking-salting process:
207 comparison between salmon and cod

208 The results obtained in the physico-chemical determinations carried out in smoke-
209 flavoured cod under different salt dose conditions are shown in Table 2. These
210 parameters were compared with those obtained for smoke-flavoured salmon by Rizo et
211 al. (2015a), who followed the same smoking-salting procedure.

212

Table 2

213

214 The smoking-salting process led to a significant reduction in moisture and an increase in
215 NaCl content, which lowered the initial a_w values. For each tested condition, smoke-
216 flavoured cod showed higher salt and moisture contents than the salmon samples. It is
217 noteworthy that at the lowest salt dose (4 g/100 g fresh fish), the z^{NaCl} and a_w values
218 were similar for both cod and salmon. This means that when using the same procedure
219 conditions to achieve a similar a_w to salmon, a higher salt concentration is required with
220 cod, given its higher moisture content.

221 When the total, water and salt weight changes in cod and salmon were compared, the
222 most important factor was fish type. The ΔM_t^o , ΔM_t^w , and ΔM_t^{NaCl} values were always
223 higher for the smoke-flavoured cod than for salmon with all the salt doses. This result
224 indicates the influence of fish characteristics on the smoking-salting process. The most
225 marked difference between both species was lipid content, which was higher in salmon
226 than in cod (Table 2). In the literature it is well-known that lipid content in fish is a
227 limiting factor during salting and drying processes, which acts as a physical barrier for
228 water diffusion and replaces the aqueous part that serves as a vector for transfer during
229 salting (Cardinal et al., 2001). Different studies have reported the strong effect of the
230 lipid phase during fish drying or brine salting (Collignan et al., 2001; Czerner et al.,

231 2013; Gallart-Jornet et al., 2007). During the salting process, the main mass transfer
232 fluxes are water and soluble solids, which occur in the fish aqueous phase (Barat et al.,
233 2003). Accordingly, fat acts as an inert component in the mass transfer. Therefore,
234 weight changes (total, water and salt weight changes) would be lower in those fish
235 species with a higher lipid content. This fact could explain why the ΔM_t^o , ΔM_t^w and
236 ΔM_t^{NaCl} values were higher in the cod samples than in the salmon ones, and
237 independently of the salt dose tested. However when these parameters were expressed
238 on a fat-free basis, the difference between the cod and salmon samples significantly
239 reduced. The statistical analysis still reported significant differences between both fish
240 species, a behaviour that has been previously described by other authors (Gallart-Jornet
241 et al., 2007).

242 After finishing the smoking-salting process, the liquid released by fish into WP bags
243 was collected and weighed. The exudate was composed mainly of water, unabsorbed
244 salt and soluble proteins (Barat et al., 2003). No exudate was observed in the smoke-
245 flavoured salmon samples, whereas a certain amount of exudate was collected from the
246 smoke-flavoured cod samples, which became higher with increasing amounts of the salt
247 dose used.

248 Zugarramurdi and Lupin (1980) developed models to predict sodium chloride uptake
249 and water loss. The capacity of these models has been proven by different authors for
250 several fish species (Bellagha et al., 2007; Boudhrioua et al., 2009; Sobukola and
251 Olatunde, 2011). In dry salting studies, Bellagha et al. (2007) observed that water
252 content decreased rapidly and the highest amount of exudate was released during the
253 first 24 hours.

254 According to the models of Zugarramurdi and Lupin (1980), water loss is dependent on
255 the initial and final moisture contents, which explains the larger amount of exudate

256 collected in WP bags after cod processing compared with salmon. The exudation rate
257 was lower for the salmon samples, and the processing conditions (RH, temperature and
258 processing time) were suitable for evaporating all the exudate. In contrast, the same
259 conditions were inappropriate for evaporating all the exudate formed during cod
260 processing with WP bags. The drying rate was directly related to air temperature,
261 velocity and RH. Therefore by modifying these parameters, it is possible to evaporate
262 water release by fish muscle. However, drying fish at very low RH can lead to
263 hardening on the surface and a lower drying rate. Case hardening is the progressive
264 formation of a thick crust of salt and protein on the surface. The crust prevents
265 migration of water and the centre of the fish can become spoiled, even though it looks
266 dried (Andrés et al., 2007). Crust formation was observed by Rizo et al. (2015a) by the
267 same smoking-salting procedure at 50% RH, therefore 60% RH was fixed as a
268 minimum value. Nevertheless, it is well-known that the temperature increase accelerates
269 moisture and salt diffusion (Bellagha et al., 2007; Boudhrioua et al., 2009).
270 Accordingly, increasing slightly the chamber temperature would result in a drop in
271 humidity, which would enhance the drying rate (Rørå et al., 2005)

272 Water activity (a_w) is directly related with the microbiological load of salted and
273 smoked products. Therefore, it can be considered a decisive parameter to ensure smoked
274 fish safety. With salted products, a high correlation exists between a_w and NaCl
275 concentration in the liquid phase (z^{NaCl}) (Fuentes et al., 2008). By taking the a_w
276 parameter as a reference to establish the processing conditions, a 4% salt dose allowed a
277 smoke-flavoured cod product to be obtained with a similar a_w value to the target ($a_w =$
278 0.965, average values of both brands) (Table 1). However, the samples processed under
279 these conditions showed a higher moisture and salt content than the reference. None of
280 the smoke-flavoured cod samples obtained in this phase of the study achieved the

281 reference moisture and salt content. For this reason, processing conditions should be
282 adjusted to obtain a smoke-flavoured cod product that has less moisture while
283 maintaining the proposed salt level. As previously mentioned, more intense moisture
284 loss can be achieved by raising the processing temperature, and by also prolonging the
285 processing time. In order to avoid crust formation on cod samples, 60% and 70% RH
286 were established in the drying chamber for smoking-salting process optimisation
287 purposes.

288

289 *3.3. Phase II. Optimisation of the smoking-salting process for cod*

290 Accordingly to these results, optimisation of the smoking-salting cod procedure entailed
291 reducing salt dosage, modifying temperature and RH in the drying chamber, and
292 prolonging the process.

293 Moisture of the smoke-flavoured cod samples significantly lowered with higher salt
294 doses, a lower RH and longer processing times (Fig. 3 and Table 4). The samples
295 processed with the highest salt dose (4%), the lowest RH (60%) and for a longer time
296 gave the lowest moisture values. The samples submitted to a smoking-salting process
297 that lasted less than 96 h did not achieve the reference moisture content, independently
298 of the salt dose level or RH.

299

Fig. 3

300

301 Salt concentration significantly increased with salt dose, RH and processing time (Fig.
302 4). Salt content was slightly lower in the samples processed at 70% RH compared with
303 those processed at 60% RH, due to the lower dehydration undergone by these samples.
304 This effect was stronger as the processing time increased. Likewise for the same salt
305 dose, a longer processing time yielded higher salt content. The samples processed with

306 the highest salt dose gave a very high final salt content, unlike the food industry trend to
307 reduce the amount of sodium in processed food. Indeed the EU has established a
308 common framework to reduce salt intake in the general population (European
309 Commission, 2009). The European approach to salt reduction focuses on a limited
310 number of food categories, which include fish products. To achieve this goal, the
311 European Commission supports research into reducing sodium in foods to the lowest
312 possible level, while maintaining food safety and consumer acceptance. Therefore, it
313 would be interesting to select processing conditions that allow a smoked fish product to
314 be obtained with the lowest salt content and an a_w value that ensures product safety.

315 **Fig. 4**

316

317 The effect of salt dose, RH and processing time on the a_w parameter was similar to that
318 observed for moisture. Thus when salt dose increased and/or RH decreased, lower a_w
319 values were detected (Figure 5).

320 **Fig. 5**

321

322 The relative mass changes and physico-chemical parameters in the smoke-flavoured cod
323 obtained by the new smoking-salting process are shown in Table 3

324 **Table 3**

325

326 The longer the processing time, the more marked the total and water weight changes
327 were (ΔM_t^o and ΔM_t^w) in higher dehydration. Regardless of salt dose, ΔM_t^o and ΔM_t^w
328 were higher in the samples processed at 60% RH than the 70% RH samples. Total
329 weight changes can be considered a combination of both weight changes (water and
330 NaCl). These data were consistent with the moisture values recorded for the same

331 conditions (Fig. 3). The modifications introduced into the processing parameters
332 allowed the moisture and a_w of the fish samples to lower, but had very little impact on
333 the salt content calculated on a dry basis (Table 3). No tendency to case hardening was
334 detected in the samples, which indicates that using 60% or 70% RH and raising the
335 temperature from 5 to 10°C were adequate for the smoking-salting process.

336 It should be noted that no exudate was obtained after the smoking-salting in the samples
337 processed for 72 h and 96 h under all the processing conditions studied. WP bags
338 allowed the complete evaporation of the water released by muscle during processing, as
339 observed in the smoking-salting of salmon with WP (Rizo et al., 2015a). These results
340 confirm the effectiveness of WP bags, and that salting, drying and smoking stages can
341 be carried out in a single step to thus reduce handling operations and processing steps
342 compared with traditional methods. Evaporation of residual brine and a controlled salt
343 dose reduce the final volume of brine wastes generated by the process. This could be a
344 great advantage as these brines are highly polluting and require expensive treatment and
345 waste disposal. Furthermore, smoking-salting inside a bag enables fish processing to
346 take place under more controlled conditions than traditional methods (unpacked) (Rizo
347 et al., 2015b).

348 The statistical analysis showed that all the factors strongly influenced each evaluated
349 parameter (Table 4). The effect of salt dose and processing time was stronger compared
350 to RH for all the considered parameters. Salt dose was the factor with the most marked
351 effect on the salt content-related variables measured (x^{NaCl} , X^{NaCl} , z^{NaCl} , and ΔM_t^{NaCl}), as
352 confirmed by the F-ratio results obtained in the statistical analysis (Table 4). The same
353 behaviour was observed for the a_w parameter, whereas processing time had a stronger
354 effect on moisture, weight loss and water weight changes. These effects can be
355 explained by the fact that the fish packaged in WP bags continued to lose moisture

356 throughout the process, while the increase in salt content ended when muscle absorbed
357 all the dosed salt. Some interactions were also detected between factors, but were
358 generally non-significant or had a minor effect on the studied parameters compared with
359 the independently analysed factors.

360

Table 4

361

362

363 **4. Conclusions**

364 Smoke-flavoured cod samples showed higher salt and moisture contents than the
365 salmon samples under the same processing conditions. In the cod samples, a higher salt
366 concentration was required to achieve similar a_w values to those of salmon, which
367 highlights the influence of lipid content during smoking-salting. The processing
368 conditions which led to complete exudate evaporation through WP bags during salmon
369 processing were insufficient to evaporate all the water released by fish muscle when the
370 process was applied to cod. Optimising processing conditions allowed exudate
371 evaporation when the process was prolonged 72 and 96 h.

372 According to the obtained results, the combination of WP bags and a 2% NaCl dose,
373 60% RH and a 96-hour processing time were the optimal conditions. These processing
374 parameters enabled us to obtain a smoke-flavoured cod product with similar a_w ,
375 moisture and salt content to the reference, but with the lowest salt dose. Nevertheless,
376 the suitability of the smoking-salting process applied to cod should be confirmed by
377 shelf-life studies and sensory evaluations.

378 These results indicate that this new methodology can be applied to obtain smoke-
379 flavoured products from different fish types by adapting processing parameters to the
380 specific features of each fish species. This new smoking-salting is a suitable alternative

381 to traditional cold-smoking procedures since it minimises brine wastes, cuts processing
382 steps and facilitates fish handling during chain production, which makes the process
383 more hygienic, simpler and faster.

384

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389

390 **6. References**

391 Andrés, A., Rodríguez-Barona, S., Barat, J.M., Fito, P. (2005). Salted cod
392 manufacturing: influence of salting procedure on process yield and product
393 characteristics. *Journal of Food Engineering*, 69(4), 467–471

394 Andrés, A., Barat, J.M., Grau, R., Fito, P. (2007). Principles of drying and smoking. In:
395 Toldrá, F., Hui, Y.H., Nip, W., Sebranek, J.G., Stahnke, L., Silveira, E.F., Talon, R.
396 (Eds.), *Handbook of Fermented Meat and Poultry*. IFT Press – Blackwell Publishing,
397 Ames, Iowa, pp. 37–50.

398 AOAC (1997). *Official methods of analysis* (16th ed.). Washington: Association of
399 Official Analytical Chemists.

400 Barat, J.M., Rodríguez-Barona, S., Andrés, A., Fito, P. (2003). Cod salting
401 manufacturing analysis. *Food Research International*, 36(5), 447–453.

402 Barat, J.M., Gallart-Jornet, L., Andrés, A., Akse, L., Carlehög, M., Skjerdal, O.T.
403 (2006). Influence of cod freshness on the salting, drying and desalting stages.
404 *Journal of Food Engineering*, 73(1), 9-19.

405 Bellagha, S., Sahli, A., Farhat, A., Kechaou, N., Glenza, A. (2007). Studies on salting
406 and drying of sardine (*Sardinella aurita*): Experimental kinetics and modelling.
407 *Journal of Food Engineering*, 78(3), 947–952

408 Boudhrioua, N., Djendoubi, N., Bellagha, S., Kechaou, N. (2009). Study of moisture
409 and salt transfers during salting of sardine fillets. *Journal of Food Engineering*, 94,
410 83–89.

411 Cardinal, M., Knockaert, C., Torrissen, O., Sigurgisladottir, S., Mørkøre, T.,
412 Thomassen, M., Vallet, J.L. (2001). Relation of smoking parameters to the yield,
413 colour and sensory quality of smoked Atlantic salmon (*Salmo salar*). *Food Research*
414 *International*, 34(6), 537–550.

415 Cardinal, M., Gunnlaugsdottir, H., Bjoernevik, M., Ouisse, A., Vallet, J.L., Leroi, F.
416 (2004). Sensory characteristics of cold-smoked Atlantic salmon (*Salmo salar*) from
417 European market and relationships with chemical, physical and microbiological
418 measurements. *Food Research International*, 37(2), 181–193.

419 Codex Alimentarius (2013). Standard for Smoked Fish, Smoke-Flavoured Fish and
420 Smoke-Dried Fish, 311–2013.

421 Cornu, M., Beaufort, A., Rudelle, S., Laloux, L., Bergis, H., Miconnet, N., Serot, T.,
422 Delignette-Muller, M.L (2006). Effect of temperature, water-phase salt and phenolic
423 contents on *Listeria monocytogenes* growth rates on cold-smoked salmon and
424 evaluation of secondary models. *International Journal of Food Microbiology*, 106,
425 159-168

426 Czerner, M., Yeannes, M.I. (2013). Modelling the effect of temperature and lipid
427 content on anchovy (*Engraulis anchoita*) salting kinetics. *Journal of Food*
428 *Engineering*, 115(2), 164–172.

- 429 Collignan, A., Bohuon, P., Deumier, F., Poligné, I. (2001). Osmotic treatment of fish
430 and meat products. *Journal of Food Engineering*, 49 (2–3), 153–162.
- 431 Espe, M., Kiessling, A., Lunestad, B., Torrissen, O.J., Rørå, A.M.B. (2004). Quality of
432 cold smoked collected in one French hypermarket during a period of 1 year.
433 *Lebensmittel-Wissenschaft und-Technologie*, 37, 627–638
- 434 European Commission (2003). Regulation 2065/2003 of November 10, 2003. Smoke
435 flavourings used or intended for use in or on foods. Official Journal of the European
436 Union, L 309/46.
- 437 European Commission (2009). National Salt Initiatives. Implementing the EU
438 Framework for salt reduction initiatives.
439 [http://ec.europa.eu/health/nutrition_physical_activity/high_level_group/nutrition_sal](http://ec.europa.eu/health/nutrition_physical_activity/high_level_group/nutrition_salt_en.htm)
440 [t_en.htm](http://ec.europa.eu/health/nutrition_physical_activity/high_level_group/nutrition_salt_en.htm) [Accessed 01.05.15]
- 441 Fuentes, A., Barat, J.M., Fernández-Segovia, I., Serra, J.A. (2008). Study of sea bass
442 (*Dicentrarchus labrax L.*) salting process: Kinetic and thermodynamic control. *Food*
443 *Control*, 19(8), 757–763.
- 444 Fuentes, A., Fernández-Segovia, I., Barat, J.M., Serra, J.A. (2010a). Physicochemical
445 Characterization of Some Smoked and Marinated Fish Products. *Journal of Food*
446 *Processing and Preservation*, 34(1), 83–103.
- 447 Fuentes, A., Fernández-Segovia, I., Serra, J.A., Barat, J.M. (2010b). Development of a
448 smoked sea bass product with partial sodium replacement. *LWT - Food Science and*
449 *Technology*, 43(9), 1426–1433.
- 450 Gallart-Jornet, L., Barat, J. M., Rustad, T., Erikson, U., Escriche, I., Fito, P. (2007). A
451 comparative study of brine salting of Atlantic cod (*Gadus morhua*) and Atlantic
452 salmon (*Salmo salar*). *Journal of Food Engineering*, 79(1), 261–270.

453 Ismail, N., Wootton, M. (1992). Fish salting and drying: a review. *ASEAN Food*
454 *Journal*, 7(4), 175–183.

455 Jittinandana, S., Kenney, P.B., Slider, S.D., Kiser, R.A. (2002). Effect of brine
456 concentration and brining time on quality of smoked rainbow trout fillets. *Journal of*
457 *Food Science*, 67, 2095–2099.

458 Jørgensen, L.V., Dalgaard, P., Huss, H.H. (2000). Multiple compound quality index for
459 cold-smoked salmon (*Salmo salar*) developed by multivariate regression of biogenic
460 amines and pH. *Journal of Agricultural and Food Chemistry*, 48(6), 2448-2453.

461 Karásková, P., Fuentes, A., Fernández-Segovia, I., Alcañiz, M., Masot, R., Barat, J.M.
462 (2011). Development of a low-cost non-destructive system for measuring moisture
463 and salt content in smoked fish products. ICEF 2011. *Procedia e Food Science*, 1,
464 1195-1201

465 Leroi, F., Joffraud, J.J., Chevalier, F., Cardinal, M. (2001). Research of quality indices
466 for cold-smoked salmon using a stepwise multiple regression of microbiological
467 counts and physico-chemical parameters. *Journal of Applied Microbiology*, 90(4),
468 578-587.

469 Mørkøre, T., Vallet, J.L., Cardinal, MC, Montero, P., Torrissen, O.J., Nortvedt, R.,
470 Sigurgisladottir, S., Thomassen, M., (2001). Fat content and fillet shape of Atlantic
471 salmon: relevance for processing yield and quality of raw and smoked products.
472 *Journal of Food Science*, 66, 1348–1354.

473 Rizo, A., Fuentes, A., Fernández-Segovia, I., Masot, R., Alcañiz, M., Barat, J.M.
474 (2013). Development of a new salmon salting–smoking method and process
475 monitoring by impedance spectroscopy. *LWT - Food Science and Technology*,
476 51(1), 218–224.

477 Rizo, A., Máñes, V., Fuentes, A., Fernández-Segovia, I., Barat, J.M. (2015a). A novel
478 process for obtaining smoke-flavoured salmon using water vapour permeable bags.
479 *Journal of Food Engineering*, 149, 44–50.

480 Rizo, A., Máñes, V., Fuentes, A., Fernández-Segovia, I., Barat, J.M. (2015b).
481 Physicochemical and microbial changes during storage of smoked-flavoured salmon
482 obtained by a new method. *Food Control*, 56, 195-201

483 Rørå, A.B., Kvåle, A., Mørkøre, T., Rørvik, K.A., Steien, S.H., Thomassen, M.S.
484 (1998). Process yield, colour and sensory quality of smoked Atlantic salmon (*Salmo*
485 *salar*) in relation to raw material characteristics. *Food Research International*,
486 31(8), 601–609.

487 Rørå, A.M.B., Birkeland, S., Hultmann, L., Rustad, T., Skåra, T., Bjerkeng, B. (2005).
488 Quality characteristics of farmed Atlantic salmon (*Salmo salar*) fed diets high in
489 soybean or fish oil as affected by cold-smoking temperature. *LWT - Food Science*
490 *and Technology*, 38, 201–211.

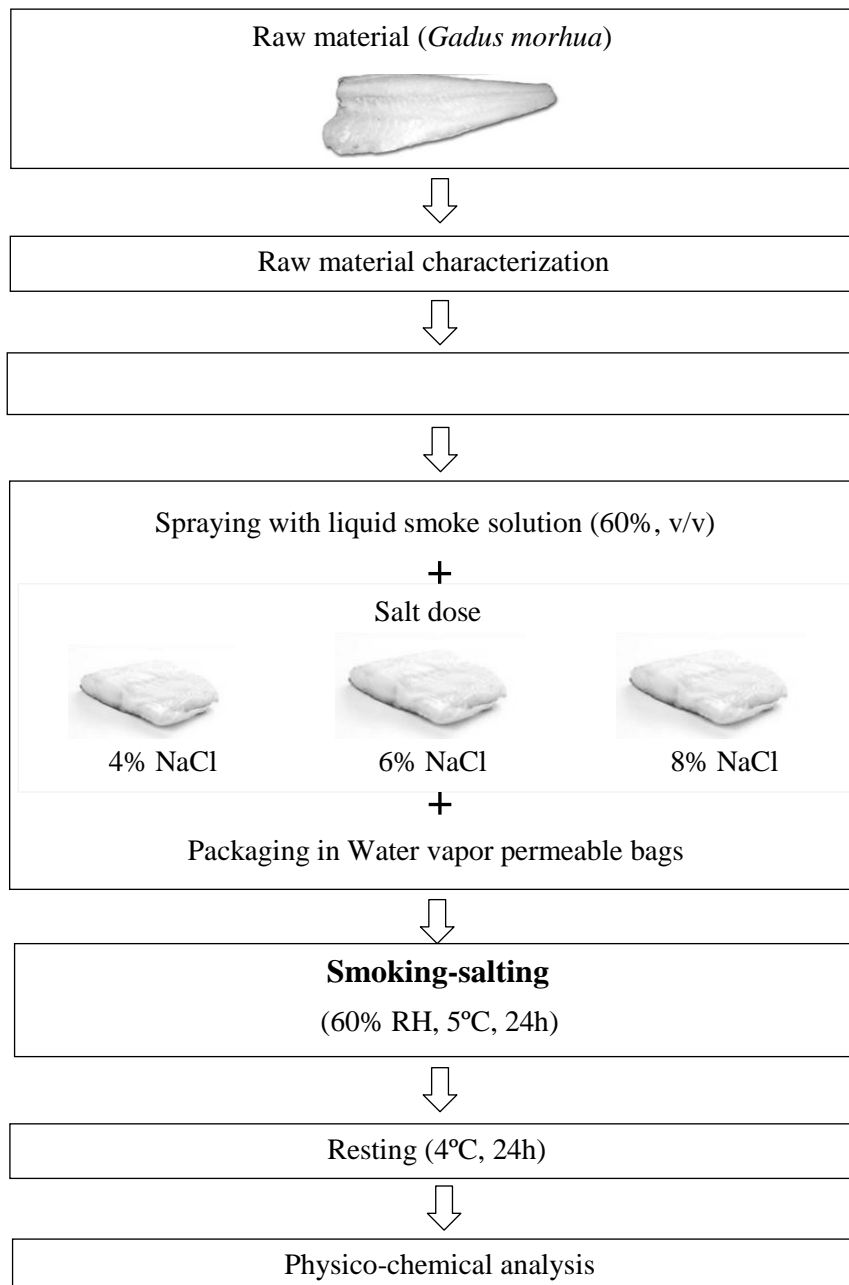
491 Sobukola, O.P., Olatunde, S.O. (2011). Effect of salting techniques on salt uptake and
492 drying kinetics of African catfish (*Clarias gariepinus*). *Food and Bioproducts*
493 *Processing*, 89(3), 170–177.

494 Wang, D., Correia, L.R., Tang, J. (1998). Modelling of salt diffusion in Atlantic salmon
495 muscle. *Canadian Agricultural Engineering*, 40(1), 29–34.

496 Wang, D., Tang, J., Correia, L.R. (2000). Salt diffusivities and salt diffusion in farmed
497 Atlantic salmon muscle as influenced by rigor mortis. *Journal of Food Engineering*
498 43, 115–123.

499 Zugarramurdi, A., Lupin, H.M. (1980). A model to explain observed behaviour on fish
500 salting. *Journal of Food Science*, 45, 1305–1317.

501

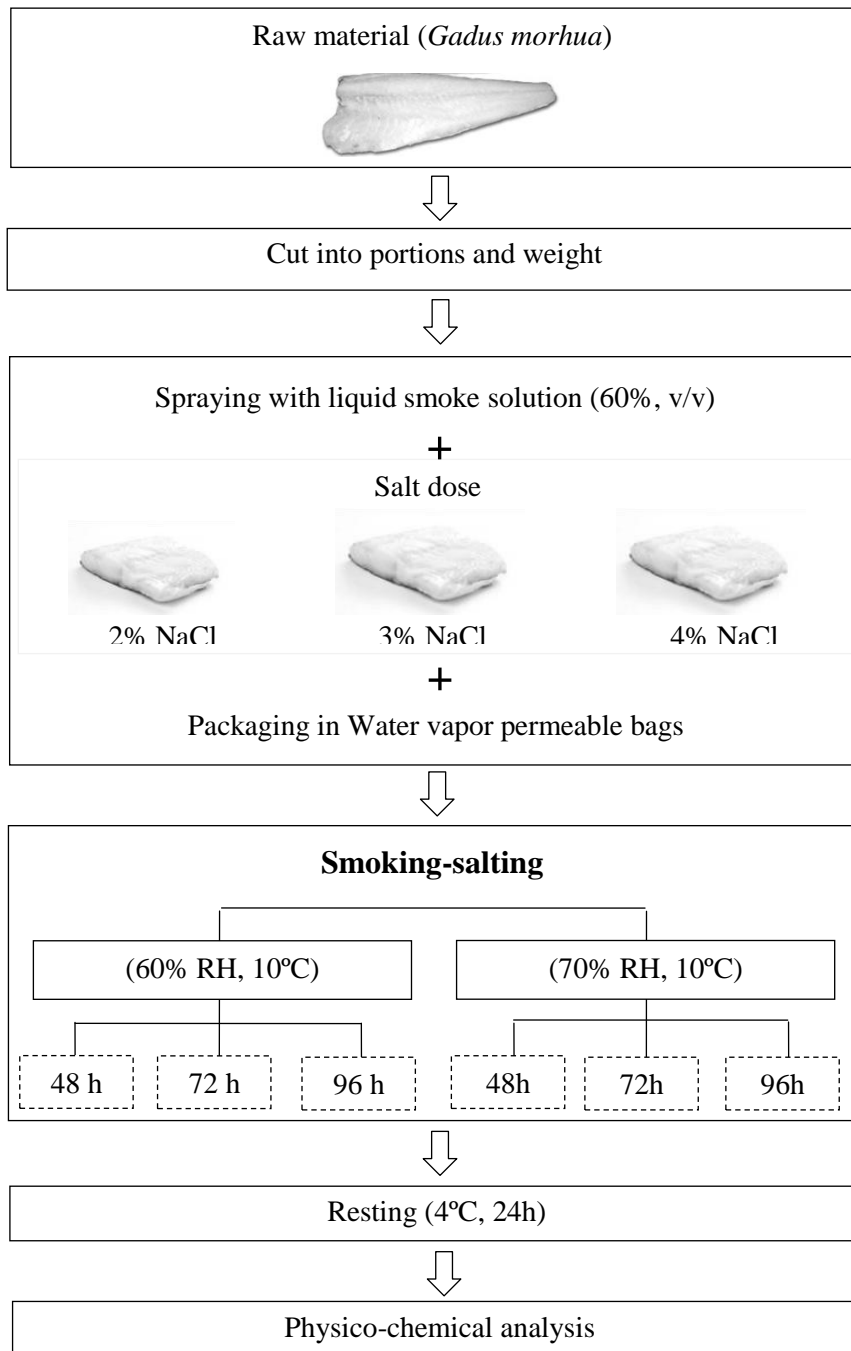


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Figure 1

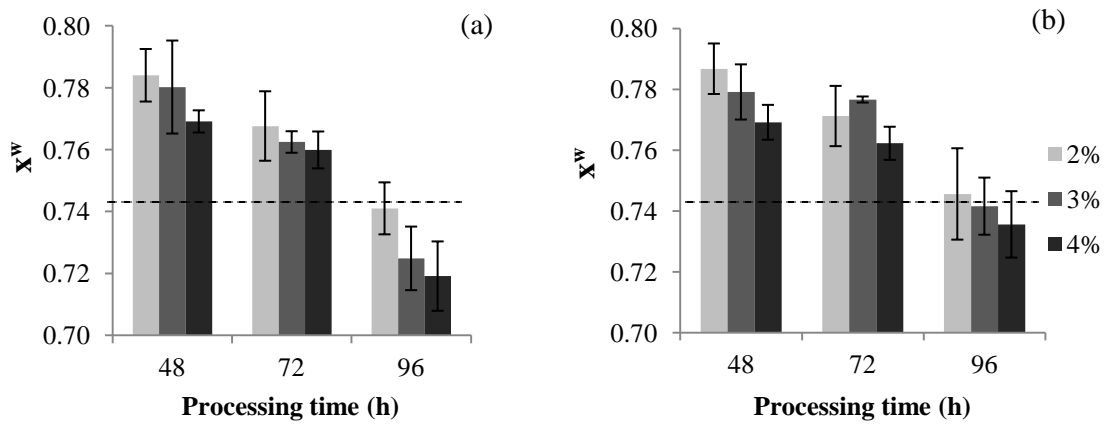


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Figure 2

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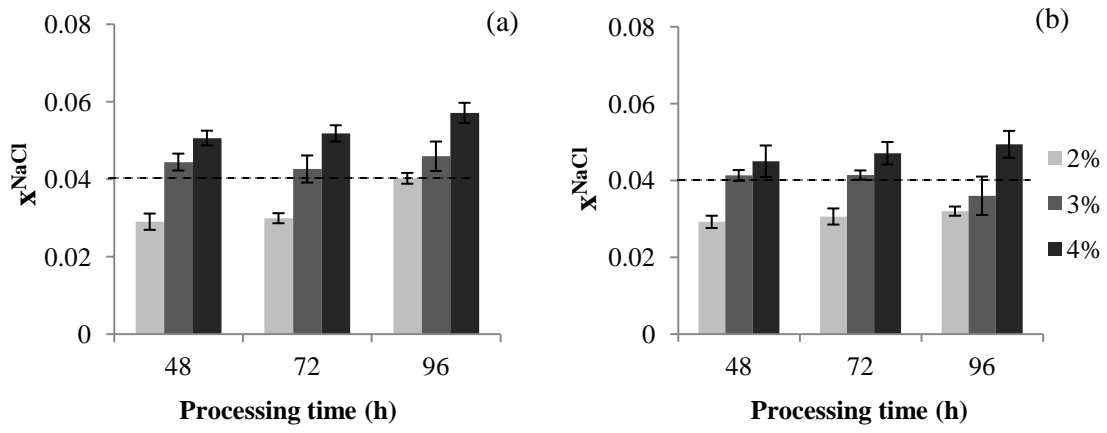
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Figure 3

Figure 4

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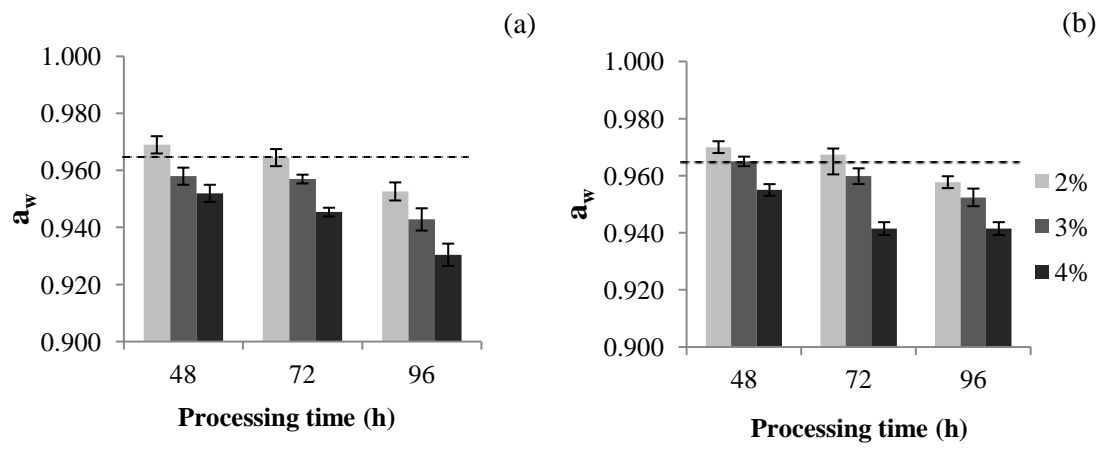
Figure 4

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Figure 5

1 **FIGURE CAPTIONS**

2 **Figure 1.** Smoking-salting process in phase I.

3

4 **Figure 2.** Smoking-salting process in phase II.

5

6 **Fig. 3.** Moisture values of the smoke-flavoured cod samples obtained using different
7 relative humidity 60% RH (a) and 70% RH (b), salt doses (2, 3, and 4% NaCl), and
8 processing times (48, 72, 96 h). Mean values \pm SD (n = 3). Bars represent the standard
9 deviation from triplicate determination. The dashed line represents the reference values.

10

11 **Fig. 4.** Salt content values of the smoke-flavoured cod samples obtained using different
12 relative humidity 60% RH (a) and 70% RH (b), salt doses (2, 3, and 4% NaCl), and
13 processing times (48, 72, 96 h). Mean values \pm SD (n = 3). Bars represent the standard
14 deviation from triplicate determination. The dashed line represents the reference values.

15

16 **Fig. 5.** Water activity values of the smoke-flavoured cod samples obtained using
17 different relative humidity 60% RH (a) and 70% RH (b), salt doses (2, 3, and 4% NaCl),
18 and processing times (48, 72, 96 h). Mean values \pm SD (n = 3). Bars represent the
19 standard deviation from triplicate determination. The dashed line represents the
20 reference values.

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22

1 **Table 1.** Moisture, lipid, salt content, salt content in the liquid phase and a_w of raw
2 material and commercial smoked cod (brand A and B). Mean values \pm SD (n=3).

3

	x^w	x^f	x^{NaCl}	z^{NaCl}	a_w
<i>Raw material</i>	0.835 \pm 0.004	0.0016 \pm 0.0009	-	-	0.994 \pm 0.003
<i>Smoked cod A</i>	0.732 \pm 0.003	0.0013 \pm 0.0005	0.053 \pm 0.0003	0.068 \pm 0.003	0.959 \pm 0.002
<i>Smoked cod B</i>	0.751 \pm 0.013	0.0012 \pm 0.0001	0.023 \pm 0.0002	0.031 \pm 0.002	0.973 \pm 0.005

4

1 **Table 2.** Physico-chemical parameters and relative mass changes in smoke-flavoured
 2 cod and salmon obtained using different salt doses (4, 6, and 8% NaCl). Mean values \pm
 3 SD (n = 3).

4

Salt dose (g/100g)	Smoke-flavoured cod			Smoke-flavoured salmon*			α	
	4	6	8	4	6	8	S	F
X^w	0.792 \pm 0.009 ^{aA}	0.771 \pm 0.009 ^{abA}	0.757 \pm 0.009 ^{bA}	0.636 \pm 0.02 ^{abB}	0.619 \pm 0.005 ^{abB}	0.613 \pm 0.029 ^{bB}	*	***
X^{NaCl}	0.045 \pm 0.004 ^{aA}	0.057 \pm 0.003 ^{aA}	0.067 \pm 0.008 ^{bA}	0.037 \pm 0.005 ^{aB}	0.035 \pm 0.004 ^{aB}	0.040 \pm 0.012 ^{bB}	*	***
X_{ff}^{NaCl}	0.207 \pm 0.031 ^{aA}	0.249 \pm 0.019 ^{aA}	0.278 \pm 0.046 ^{aA}	0.103 \pm 0.021 ^{aB}	0.092 \pm 0.009 ^{aB}	0.103 \pm 0.035 ^{aB}	ns	***
X_{ff}^{NaCl}	0.207 \pm 0.031 ^{aA}	0.249 \pm 0.019 ^{aA}	0.278 \pm 0.046 ^{aA}	0.130 \pm 0.014 ^{aB}	0.127 \pm 0.014 ^{aB}	0.131 \pm 0.010 ^{aB}	ns	***
Z^{NaCl}	0.056 \pm 0.004 ^{aA}	0.069 \pm 0.003 ^{aA}	0.082 \pm 0.008 ^{bA}	0.055 \pm 0.009 ^{aB}	0.054 \pm 0.006 ^{aB}	0.062 \pm 0.007 ^{bB}	**	**
a_w	0.962 \pm 0.004 ^{aA}	0.939 \pm 0.002 ^{bA}	0.931 \pm 0.006 ^{cA}	0.959 \pm 0.006 ^{aB}	0.960 \pm 0.003 ^{bB}	0.950 \pm 0.009 ^{cB}	***	***
X^f	0.002 \pm 0.001 ^{aA}	0.002 \pm 0.001 ^{aA}	0.002 \pm 0.001 ^{aA}	0.090 \pm 0.004 ^{aB}	0.104 \pm 0.005 ^{aB}	0.094 \pm 0.002 ^{aB}	ns	**
Exudate (w/w)	0.019 \pm 0.007 ^a	0.027 \pm 0.003 ^b	0.045 \pm 0.0018 ^c	-	-	-	***	-
Weight changes on wet basis								
ΔM_t^o	-0.120 \pm 0.033 ^{aA}	-0.116 \pm 0.024 ^{aA}	-0.125 \pm 0.020 ^{aA}	-0.070 \pm 0.013 ^{aB}	-0.085 \pm 0.008 ^{abB}	-0.085 \pm 0.012 ^{abB}	ns	**
ΔM_t^w	-0.147 \pm 0.028 ^{aA}	-0.154 \pm 0.015 ^{abA}	-0.157 \pm 0.012 ^{bA}	-0.071 \pm 0.014 ^{aB}	-0.096 \pm 0.009 ^{abB}	-0.102 \pm 0.012 ^{bB}	ns	***
ΔM_t^{NaCl}	0.034 \pm 0.005 ^{aA}	0.045 \pm 0.001 ^{aA}	0.055 \pm 0.006 ^{bA}	0.031 \pm 0.005 ^{aB}	0.030 \pm 0.004 ^{aB}	0.035 \pm 0.010 ^{bB}	**	***
Weight changes on fat free basis								
ΔM_{tff}^o	-0.120 \pm 0.033 ^{aA}	-0.116 \pm 0.024 ^{aA}	-0.125 \pm 0.020 ^{aA}	-0.083 \pm 0.015 ^{aA}	-0.119 \pm 0.016 ^{aA}	-0.106 \pm 0.015 ^{aA}	ns	ns
ΔM_{tff}^w	-0.147 \pm 0.028 ^{aA}	-0.153 \pm 0.015 ^{abA}	-0.157 \pm 0.012 ^{bA}	-0.074 \pm 0.015 ^{aB}	-0.109 \pm 0.011 ^{abB}	-0.108 \pm 0.012 ^{bB}	ns	***
ΔM_{tff}^{NaCl}	0.034 \pm 0.005 ^{aA}	0.045 \pm 0.001 ^{aA}	0.055 \pm 0.006 ^{bA}	0.032 \pm 0.005 ^{aB}	0.030 \pm 0.003 ^{aB}	0.036 \pm 0.003 ^{bB}	**	***

5 *Data obtained from a previous study (Rizo et al., 2015a)

6 Different lower-case letters indicate significant differences for salt dose factor (S). Different capital letters indicate
7 significant differences for fish species (F). ns: no significant, * p <0.05, ** p <0.01, *** p <0.001

1 **Table 3.** Relative mass changes and physico-chemical parameters in smoke-flavoured
 2 cod obtained using different salt doses (S), relative humidity (RH), and processing times
 3 (t). Mean values \pm SD (n = 3).

4

S (g salt/100g)	RH (%)	t (h)	ΔM_t^o	ΔM_t^w	ΔM_t^{NaCl}	X^{NaCl}	Z^{NaCl}	Exudate (g/g)
2		48	-0.240 \pm 0.051	-0.253 \pm 0.043	0.016 \pm 0.001	0.134 \pm 0.005	0.036 \pm 0.003	0.001 \pm 0.0005
3	60	48	-0.219 \pm 0.012	-0.226 \pm 0.018	0.029 \pm 0.002	0.203 \pm 0.017	0.054 \pm 0.002	0.003 \pm 0.002
4		48	-0.230 \pm 0.034	-0.244 \pm 0.028	0.034 \pm 0.003	0.219 \pm 0.011	0.062 \pm 0.002	0.008 \pm 0.002
2		72	-0.276 \pm 0.015	-0.280 \pm 0.023	0.016 \pm 0.001	0.128 \pm 0.005	0.037 \pm 0.002	-
3	60	72	-0.271 \pm 0.041	-0.280 \pm 0.031	0.025 \pm 0.002	0.180 \pm 0.005	0.053 \pm 0.002	-
4		72	-0.260 \pm 0.019	-0.273 \pm 0.012	0.033 \pm 0.002	0.216 \pm 0.014	0.064 \pm 0.002	-
2		96	-0.371 \pm 0.032	-0.381 \pm 0.023	0.018 \pm 0.001	0.150 \pm 0.004	0.052 \pm 0.003	-
3	60	96	-0.374 \pm 0.013	-0.382 \pm 0.014	0.023 \pm 0.003	0.167 \pm 0.018	0.059 \pm 0.004	-
4		96	-0.375 \pm 0.009	-0.386 \pm 0.012	0.032 \pm 0.003	0.204 \pm 0.012	0.074 \pm 0.003	-
2		48	-0.187 \pm 0.040	-0.213 \pm 0.036	0.018 \pm 0.001	0.137 \pm 0.003	0.036 \pm 0.002	0.013 \pm 0.002
3	70	48	-0.184 \pm 0.019	-0.200 \pm 0.015	0.029 \pm 0.002	0.187 \pm 0.010	0.052 \pm 0.002	0.013 \pm 0.003
4		48	-0.184 \pm 0.021	-0.213 \pm 0.025	0.031 \pm 0.003	0.196 \pm 0.020	0.056 \pm 0.007	0.020 \pm 0.002
2		72	-0.221 \pm 0.010	-0.235 \pm 0.010	0.017 \pm 0.002	0.134 \pm 0.010	0.038 \pm 0.003	-
3	70	72	-0.219 \pm 0.024	-0.229 \pm 0.020	0.027 \pm 0.001	0.185 \pm 0.004	0.051 \pm 0.002	-
4		72	-0.211 \pm 0.030	-0.234 \pm 0.020	0.034 \pm 0.001	0.197 \pm 0.018	0.058 \pm 0.003	-
2		96	-0.303 \pm 0.020	-0.316 \pm 0.012	0.015 \pm 0.002	0.130 \pm 0.004	0.041 \pm 0.001	-
3	70	96	-0.284 \pm 0.034	-0.304 \pm 0.033	0.020 \pm 0.002	0.140 \pm 0.018	0.046 \pm 0.006	-
4		96	-0.276 \pm 0.049	-0.303 \pm 0.036	0.031 \pm 0.002	0.198 \pm 0.005	0.064 \pm 0.002	-

5

1 **Table 4.** F-ratio values and significance levels obtained in multifactor ANOVA for the
 2 physico-chemical parameters according to the factors: salt dose (S), relative humidity
 3 (RH), processing time (t) and their respective two-way interactions.

4

	S	RH	t	S x RH	S x t	RH x t
x^w	10.19 ^{**}	7.25 [*]	111.85 ^{***}	0.58 ^{ns}	0.67 ^{ns}	1.85 ^{ns}
a_w	238.99 ^{***}	33.22 ^{***}	153.17 ^{***}	2.44 ^{ns}	2.75 [*]	10.18 ^{***}
x^{NaCl}	215.57 ^{***}	35.67 ^{***}	8.68 ^{***}	2.31 ^{ns}	5.81 [*]	8.39 [*]
X^{NaCl}	113.45 ^{***}	11.73 ^{**}	5.89 ^{**}	1.96 ^{ns}	5.70 ^{**}	2.33 ^{ns}
z^{NaCl}	228.18 ^{***}	41.35 ^{***}	22.94 ^{***}	2.04 ^{ns}	4.81 ^{**}	11.22 ^{***}
ΔM_t^o	1.02 ^{ns}	76.09 ^{***}	109.63 ^{***}	0.12 ^{ns}	3.76 [*]	0.16 ^{ns}
ΔM_t^w	1.44 ^{ns}	9.03 ^{**}	17.41 ^{***}	2.93 ^{ns}	0.20 ^{ns}	7.12 ^{**}
ΔM_t^{NaCl}	235.01 ^{***}	0.63 ^{ns}	8.90 ^{***}	0.13 ^{ns}	3.45 [*]	1.71 ^{ns}
Exudate (w/w)	0.86 ^{ns}	12.99 ^{***}	49.40 ^{***}	0.68 ^{ns}	0.86 ^{ns}	12.68 ^{***}

5 ns: no significant, ^{*} p <0.05, ^{**} p <0.01, ^{***} p <0.001

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7