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

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Abstract

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

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

In order to meet food industry and consumer requirements, Rizo et al. (2013) proposed a new process to obtain smoke-flavoured salmon based on the combination of a controlled salting process with smoked-flavoured salt and vacuum packaging. This methodology was able to accelerate NaCl absorption and dehydration, reducing the total processing time without affecting physico-chemical parameters compared with traditional smoked salmon. In another study, Rizo et al. (2015a) proposed applying water vapour permeable (WP) bags to improve the previously described smoking-salting method. This methodology consists in simultaneous smoking-salting in WP bags under established temperature and humidity conditions to control product dehydration. This procedure allows smoke-flavoured salmon to be obtained with not only similar physico-chemical traits and sensory acceptance to the smoked products obtained by traditional methods, but also with good hygienic quality under cold storage (Rizo et al., 2015a, b). The use of this new methodology could substitute traditional cold smoking of
fish because the physico-chemical properties, consumer acceptance and final product safety are not affected, it minimises product handling and brine wastes, and reduces the number of processing steps.

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

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

2. Material and Methods

2.1. Materials

The fish employed as raw material were frozen cod (Gadus morhua) fillets of the 1.2-1.4 kg commercial size (Alimentos Friorizados, S.A., Barcelona, Spain), which is the commonest fish state used by the fish industry. Before processing, frozen cod fillets (n=15) were thawed at 4°C for 24 h, trimmed to remove any bones and fins, and cut into
4-cm portions to obtain 5-6 portions per fillet (72 samples were obtained). The average weight and thickness of fish portions were 136±23 g and 3 cm, respectively.

The sodium chloride used for the smoking-salting process was supplied by Panreac Química, S.A (Barcelona, Spain), and the natural liquid smoke HARDWOOD AFS 10 by Amcan ingredient Ltd. (Le Chesnay, France). Water vapour permeable bags were supplied by TUB-EX ApS (Taars, Denmark) (size: 200×300×0.04 mm; water vapour transmission rate: 5000g/50µ/m²/24h (38°C/50% RH)). To determine the target physico-chemical parameters for the new product, two different commercial smoked cod brands were used. From each brand, two different batches were analysed. These products were purchased from a local market in the city of Valencia (east Spain) and had been processed by traditional cold-smoking techniques: dry salting, followed by a smoking step in a smoking chamber.

2.2. Experimental design

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

Cod portions were subjected to a simultaneous smoking-salting process (Fig. 1) following the method developed by Rizo et al. (2015a) to obtain smoke-flavoured fish. Fish samples were smoked by spraying the fillet surface with diluted liquid smoke (60 mL/100 mL solution) for 30 s. After applying liquid smoke, salting was carried out by dosing a previously established amount of NaCl on the fillet surface. Three salt dose concentrations were considered: 4, 6, and 8 g salt/100 g fresh fish. The amount of salt added to each sample was calculated from the initial fish portion weight and the initial water weight fraction (\(x_w\)) according to the procedure by Fuentes et al. (2008). Samples were introduced into WP bags and were vacuum-packaged with a vacuum packaging
machine (Tecnotrip EV-25-CD, Barcelona, Spain). It should be noted that vacuum packaging was used merely to ensure good initial contact between fish and the WP bag. Then portions were processed in a drying chamber (Binder mod. KBF Tuttlingen, Germany) at 60% RH for 24 h at 5ºC. At the end of the processing time, samples were removed from the bags and the exudate formed during the process was weighed. Fish samples were introduced into saturated brine under constant stirring for 30 s to remove any traces of salt attached to the surface. Finally, they were dried with absorbent paper, weighed and left at 4ºC for 24 h to ensure homogeneous salt distribution on the pieces.

Analyses of moisture, lipid content, NaCl content and $a_w$ were carried out on the fresh fish and the final product. Values considered as references were obtained from the commercial smoked cod analysed (moisture, salt content and $a_w$).

Fig. 1

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

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

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

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

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

\[
X^{NaCl} = \left( \frac{x^{NaCl}}{1-x^w} \right) 
\]

\[
X^f_{ff}^{NaCl} = \left( \frac{x^{NaCl}}{1-x^w-x^l} \right) 
\]

\[
z^{NaCl} = \left( \frac{x^{NaCl}}{x^w+x^{NaCl}} \right) 
\]

The total, water and sodium chloride weight changes ($\Delta M^o_i$, $\Delta M^w_i$ and $\Delta M^{NaCl}_i$ respectively) of the fish samples were calculated by Eqs. (4)-(6).

\[
\Delta M^o_i = \left( \frac{M^o_i-M^o_0}{M^o_0} \right) 
\]

\[
\Delta M^w_i = \left( \frac{M^w_i-x^w_i-M^o_0-x^w_0}{M^o_0} \right) 
\]
\[ \Delta M_t^{\text{NaCl}} = \left( \frac{M_t - M_0}{M_0} \right) \times 100 \]  

(6)

2.4. Statistical analysis

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

3. Results and Discussion

3.1 Characterisation of raw material and commercial smoked fish

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

<table>
<thead>
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<th>Table 1</th>
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| The values obtained in the physico-chemical characterisation of the raw material were similar to those reported by other authors for fresh cod (Andrés et al., 2005; Gallart-Jornet et al., 2007). In our study, the moisture and the lipid content of raw material was 83.55% and 0.16%, respectively. Compared with the other fish species used traditionally for smoking, such as salmon, herring or sardine, cod has a very low fat content, and therefore high moisture content. In salting processes, salt uptake depends on many factors: the salting method, salt dose, freezing and thawing, and on intrinsic
fish factors like fish species, muscle thickness, postmortem state and composition (Fuentes et al., 2008; Gallart et al., 2007; Jittinandana et al., 2002; Rørå et al., 1998; Wang et al., 1998). For this reason, raw material characterisation prior to adjusting smoking-salting conditions is essential (Barat et al., 2006).

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

Given the wide variability found between brands, establishing single reference values for moisture, salt and \( a_w \) is difficult. According to the Codex standard for smoked fish, smoke-flavoured fish and smoked-dried fish (Codex, 2013), 5% aqueous phase salt (\( z_{NaCl} = 0.05 \)) would be required for smoke-flavoured fish in which smoke flavour is provided by artificial flavour blends to provide complete protection against Clostridium botulinum at temperatures between 3°C and 10°C. To fulfil this standard, the target value selected for sodium chloride content in this study was \( x_{NaCl} = 0.04 \), which corresponds to \( z_{NaCl} = 0.05 \) according to a moisture value of 74.2% (the average moisture of both brands).
3.2. Phase I. Studying the feasibility of using a new smoking-salting process: comparison between salmon and cod

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

<table>
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<th>Table 2</th>
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| The smoking-salting process led to a significant reduction in moisture and an increase in NaCl content, which lowered the initial $a_w$ values. For each tested condition, smoke-flavoured cod showed higher salt and moisture contents than the salmon samples. It is noteworthy that at the lowest salt dose (4 g/100 g fresh fish), the $z_{NaCl}$ and $a_w$ values were similar for both cod and salmon. This means that when using the same procedure conditions to achieve a similar $a_w$ to salmon, a higher salt concentration is required with cod, given its higher moisture content. When the total, water and salt weight changes in cod and salmon were compared, the most important factor was fish type. The $\Delta M_0^w$, $\Delta M_t^w$, and $\Delta M_t^{NaCl}$ values were always higher for the smoke-flavoured cod than for salmon with all the salt doses. This result indicates the influence of fish characteristics on the smoking-salting process. The most marked difference between both species was lipid content, which was higher in salmon than in cod (Table 2). In the literature it is well-known that lipid content in fish is a limiting factor during salting and drying processes, which acts as a physical barrier for water diffusion and replaces the aqueous part that serves as a vector for transfer during salting (Cardinal et al., 2001). Different studies have reported the strong effect of the lipid phase during fish drying or brine salting (Collignan et al., 2001; Czerner et al., ...
During the salting process, the main mass transfer fluxes are water and soluble solids, which occur in the fish aqueous phase (Barat et al., 2003). Accordingly, fat acts as an inert component in the mass transfer. Therefore, weight changes (total, water and salt weight changes) would be lower in those fish species with a higher lipid content. This fact could explain why the $\Delta M_w^0$, $\Delta M_t^w$ and $\Delta M_t^{NaCl}$ values were higher in the cod samples than in the salmon ones, and independently of the salt dose tested. However when these parameters were expressed on a fat-free basis, the difference between the cod and salmon samples significantly reduced. The statistical analysis still reported significant differences between both fish species, a behaviour that has been previously described by other authors (Gallart-Jornet et al., 2007).

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

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

According to the models of Zugarramurdi and Lupin (1980), water loss is dependent on the initial and final moisture contents, which explains the larger amount of exudate
collected in WP bags after cod processing compared with salmon. The exudation rate was lower for the salmon samples, and the processing conditions (RH, temperature and processing time) were suitable for evaporating all the exudate. In contrast, the same conditions were inappropriate for evaporating all the exudate formed during cod processing with WP bags. The drying rate was directly related to air temperature, velocity and RH. Therefore by modifying these parameters, it is possible to evaporate water release by fish muscle. However, drying fish at very low RH can lead to hardening on the surface and a lower drying rate. Case hardening is the progressive formation of a thick crust of salt and protein on the surface. The crust prevents migration of water and the centre of the fish can become spoiled, even though it looks dried (Andrés et al., 2007). Crust formation was observed by Rizo et al. (2015a) by the same smoking-salting procedure at 50% RH, therefore 60% RH was fixed as a minimum value. Nevertheless, it is well-known that the temperature increase accelerates moisture and salt diffusion (Bellagha et al., 2007; Boudhrioua et al., 2009). Accordingly, increasing slightly the chamber temperature would result in a drop in humidity, which would enhance the drying rate (Rørå et al., 2005)

Water activity ($a_w$) is directly related with the microbiological load of salted and smoked products. Therefore, it can be considered a decisive parameter to ensure smoked fish safety. With salted products, a high correlation exists between $a_w$ and NaCl concentration in the liquid phase ($z_{NaCl}$) (Fuentes et al., 2008). By taking the $a_w$ parameter as a reference to establish the processing conditions, a 4% salt dose allowed a smoke-flavoured cod product to be obtained with a similar $a_w$ value to the target ($a_w = 0.965$, average values of both brands) (Table 1). However, the samples processed under these conditions showed a higher moisture and salt content than the reference. None of the smoke-flavoured cod samples obtained in this phase of the study achieved the
reference moisture and salt content. For this reason, processing conditions should be adjusted to obtain a smoke-flavoured cod product that has less moisture while maintaining the proposed salt level. As previously mentioned, more intense moisture loss can be achieved by raising the processing temperature, and by also prolonging the processing time. In order to avoid crust formation on cod samples, 60% and 70% RH were established in the drying chamber for smoking-salting process optimisation purposes.

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

Accordingly to these results, optimisation of the smoking-salting cod procedure entailed reducing salt dosage, modifying temperature and RH in the drying chamber, and prolonging the process. Moisture of the smoke-flavoured cod samples significantly lowered with higher salt doses, a lower RH and longer processing times (Fig. 3 and Table 4). The samples processed with the highest salt dose (4%), the lowest RH (60%) and for a longer time gave the lowest moisture values. The samples submitted to a smoking-salting process that lasted less than 96 h did not achieve the reference moisture content, independently of the salt dose level or RH.

Salt concentration significantly increased with salt dose, RH and processing time (Fig. 4). Salt content was slightly lower in the samples processed at 70% RH compared with those processed at 60% RH, due to the lower dehydration undergone by these samples. This effect was stronger as the processing time increased. Likewise for the same salt dose, a longer processing time yielded higher salt content. The samples processed with
the highest salt dose gave a very high final salt content, unlike the food industry trend to reduce the amount of sodium in processed food. Indeed the EU has established a common framework to reduce salt intake in the general population (European Commission, 2009). The European approach to salt reduction focuses on a limited number of food categories, which include fish products. To achieve this goal, the European Commission supports research into reducing sodium in foods to the lowest possible level, while maintaining food safety and consumer acceptance. Therefore, it would be interesting to select processing conditions that allow a smoked fish product to be obtained with the lowest salt content and an $a_w$ value that ensures product safety.

![Fig. 4](image)

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

![Fig. 5](image)

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

![Table 3](image)

The longer the processing time, the more marked the total and water weight changes were ($\Delta M_t^o$ and $\Delta M_t^w$) in higher dehydration. Regardless of salt dose, $\Delta M_t^o$ and $\Delta M_t^w$ were higher in the samples processed at 60% RH than the 70% RH samples. Total weight changes can be considered a combination of both weight changes (water and NaCl). These data were consistent with the moisture values recorded for the same
conditions (Fig. 3). The modifications introduced into the processing parameters allowed the moisture and $a_w$ of the fish samples to lower, but had very little impact on the salt content calculated on a dry basis (Table 3). No tendency to case hardening was detected in the samples, which indicates that using 60% or 70% RH and raising the temperature from 5 to 10ºC were adequate for the smoking-salting process. It should be noted that no exudate was obtained after the smoking-salting in the samples processed for 72 h and 96 h under all the processing conditions studied. WP bags allowed the complete evaporation of the water released by muscle during processing, as observed in the smoking-salting of salmon with WP (Rizo et al., 2015a). These results confirm the effectiveness of WP bags, and that salting, drying and smoking stages can be carried out in a single step to thus reduce handling operations and processing steps compared with traditional methods. Evaporation of residual brine and a controlled salt dose reduce the final volume of brine wastes generated by the process. This could be a great advantage as these brines are highly polluting and require expensive treatment and waste disposal. Furthermore, smoking-salting inside a bag enables fish processing to take place under more controlled conditions than traditional methods (unpacked) (Rizo et al., 2015b).

The statistical analysis showed that all the factors strongly influenced each evaluated parameter (Table 4). The effect of salt dose and processing time was stronger compared to RH for all the considered parameters. Salt dose was the factor with the most marked effect on the salt content-related variables measured ($x^\text{NaCl}$, $x^\text{NaCl}$, $z^\text{NaCl}$, and $\Delta M^\text{NaCl}$), as confirmed by the F-ratio results obtained in the statistical analysis (Table 4). The same behaviour was observed for the $a_w$ parameter, whereas processing time had a stronger effect on moisture, weight loss and water weight changes. These effects can be explained by the fact that the fish packaged in WP bags continued to lose moisture
throughout the process, while the increase in salt content ended when muscle absorbed all the dosed salt. Some interactions were also detected between factors, but were generally non-significant or had a minor effect on the studied parameters compared with the independently analysed factors.

| Table 4 |

4. Conclusions

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

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

These results indicate that this new methodology can be applied to obtain smoke-flavoured products from different fish types by adapting processing parameters to the specific features of each fish species. This new smoking-salting is a suitable alternative
to traditional cold-smoking procedures since it minimises brine wastes, cuts processing steps and facilitates fish handling during chain production, which makes the process more hygienic, simpler and faster.

5. Acknowledges

The authors gratefully acknowledge Tub-Ex Aps (Taars, Denmark) for the supply of the water vapour permeable bags and for providing all the necessary information about their use. Author A. Rizo is grateful to Universitat Politècnica de Valencia for a FPI grant.

6. References


Figure 1

1. Raw material (Gadus morhua)

2. Raw material characterization

3. Spraying with liquid smoke solution (60%, v/v)
   - Salt dose
     - 4% NaCl
     - 6% NaCl
     - 8% NaCl
   - Packaging in Water vapor permeable bags

4. Smoking-salting
   - (60% RH, 5°C, 24h)

5. Resting (4°C, 24h)

6. Physico-chemical analysis

Figure 1
Figure 2

Raw material (*Gadus morhua*)

↓

Cut into portions and weight

↓

Spraying with liquid smoke solution (60%, v/v)

+ Salt dose

<table>
<thead>
<tr>
<th>Salt dose</th>
<th>2% NaCl</th>
<th>3% NaCl</th>
<th>4% NaCl</th>
</tr>
</thead>
</table>

Packaging in Water vapor permeable bags

↓

**Smoking-salting**

(60% RH, 10°C)

48 h 72 h 96 h

(70% RH, 10°C)

48 h 72 h 96 h

↓

Resting (4°C, 24h)

↓

Physico-chemical analysis

Figure 2
Figure 3

(a) Processing time (h)

(b) Processing time (h)
Figure 4

(a)

(b)

Processing time (h)

Processing time (h)

Figure 4
FIGURE CAPTIONS

Figure 1. Smoking-salting process in phase I.

Figure 2. Smoking-salting process in phase II.

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

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

Fig. 5. Water activity values of the smoke-flavoured cod samples obtained using different relative humidity 60% RH (a) and 70% RH (b), salt doses (2, 3, and 4% NaCl), and processing times (48, 72, 96 h). Mean values ± SD (n = 3). Bars represent the standard deviation from triplicate determination. The dashed line represents the reference values.
Table 1. Moisture, lipid, salt content, salt content in the liquid phase and $a_w$ of raw material and commercial smoked cod (brand A and B). Mean values ± SD (n=3).

<table>
<thead>
<tr>
<th></th>
<th>$x^w$</th>
<th>$x^l$</th>
<th>$x^{NaCl}$</th>
<th>$z^{NaCl}$</th>
<th>$a_w$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw material</td>
<td>0.835±0.004</td>
<td>0.0016±0.0009</td>
<td>-</td>
<td>-</td>
<td>0.994±0.003</td>
</tr>
<tr>
<td>Smoked cod A</td>
<td>0.732±0.003</td>
<td>0.0013±0.0005</td>
<td>0.053±0.0003</td>
<td>0.068±0.003</td>
<td>0.959±0.002</td>
</tr>
<tr>
<td>Smoked cod B</td>
<td>0.751±0.013</td>
<td>0.0012±0.0001</td>
<td>0.023±0.0002</td>
<td>0.031±0.002</td>
<td>0.973±0.005</td>
</tr>
</tbody>
</table>
Table 2. Physico-chemical parameters and relative mass changes in smoke-flavoured cod and salmon obtained using different salt doses (4, 6, and 8% NaCl). Mean values ± SD (n = 3).

<table>
<thead>
<tr>
<th>Salt dose (g/100g)</th>
<th>Smoke-flavoured cod</th>
<th>Smoke-flavoured salmon</th>
<th>α</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4</td>
<td>6</td>
<td>8</td>
</tr>
<tr>
<td>$X^w$</td>
<td>0.792±0.009**</td>
<td>0.771±0.009**</td>
<td>0.757±0.010**</td>
</tr>
<tr>
<td>$X^{NaCl}$</td>
<td>0.045±0.004**</td>
<td>0.057±0.005**</td>
<td>0.067±0.006**</td>
</tr>
<tr>
<td>$a_w$</td>
<td>0.207±0.031**</td>
<td>0.249±0.019**</td>
<td>0.278±0.046**</td>
</tr>
<tr>
<td>$X^{NaCl}$</td>
<td>0.207±0.031**</td>
<td>0.249±0.019**</td>
<td>0.278±0.046**</td>
</tr>
<tr>
<td>$X^{NaCl}$</td>
<td>0.056±0.004**</td>
<td>0.069±0.006**</td>
<td>0.082±0.008**</td>
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<tr>
<td>$X_f$</td>
<td>0.962±0.004**</td>
<td>0.939±0.005**</td>
<td>0.931±0.005**</td>
</tr>
<tr>
<td>$X_{exudate (w/w)}$</td>
<td>0.019±0.007**</td>
<td>0.027±0.003**</td>
<td>0.045±0.0018**</td>
</tr>
</tbody>
</table>

Weight changes on wet basis

| $\Delta M_i^w$ | -0.120±0.033** | -0.116±0.024** | -0.125±0.020** | -0.070±0.013** | -0.085±0.012** | -0.085±0.012** |    |   |
| $\Delta M_{fNaCl}$ | -0.147±0.028** | -0.154±0.015** | -0.157±0.012** | -0.071±0.014** | -0.096±0.011** | -0.102±0.012** |    |   |
| $\Delta M_{fNaCl}$ | 0.034±0.005** | 0.045±0.001** | 0.055±0.006** | 0.031±0.005** | 0.030±0.004** | 0.035±0.010** |    |   |

Weight changes on fat free basis

| $\Delta M_i^{ff}$ | -0.120±0.033** | -0.116±0.024** | -0.125±0.020** | -0.083±0.015** | -0.119±0.016** | -0.106±0.015** |    |   |
| $\Delta M_{fNaCl}$ | -0.147±0.028** | -0.153±0.015** | -0.157±0.012** | -0.074±0.015** | -0.109±0.011** | -0.108±0.012** |    |   |
| $\Delta M_{fNaCl}$ | 0.034±0.005** | 0.045±0.001** | 0.055±0.006** | 0.032±0.005** | 0.030±0.003** | 0.036±0.003** |    |   |

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

Different lower-case letters indicate significant differences for salt dose factor (S). Different capital letters indicate significant differences for fish species (F). ns: no significant, * p <0.05, ** p <0.01, *** p <0.001
Table 3. Relative mass changes and physico-chemical parameters in smoke-flavoured cod obtained using different salt doses (S), relative humidity (RH), and processing times (t). Mean values ± SD (n = 3).

<table>
<thead>
<tr>
<th>S (g salt/100g)</th>
<th>RH (%)</th>
<th>t (h)</th>
<th>ΔM₀</th>
<th>ΔMᵢ</th>
<th>ΔMᵢNaCl</th>
<th>XNaCl²</th>
<th>zNaCl²</th>
<th>Exudate (g/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>48</td>
<td>-0.240±0.051</td>
<td>-0.253±0.043</td>
<td>0.016±0.001</td>
<td>0.134±0.005</td>
<td>0.036±0.003</td>
<td>0.001±0.0005</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>60</td>
<td>-0.219±0.012</td>
<td>-0.226±0.018</td>
<td>0.029±0.002</td>
<td>0.203±0.017</td>
<td>0.054±0.002</td>
<td>0.003±0.002</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>48</td>
<td>-0.230±0.034</td>
<td>-0.244±0.028</td>
<td>0.034±0.003</td>
<td>0.219±0.011</td>
<td>0.062±0.002</td>
<td>0.008±0.002</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>72</td>
<td>-0.276±0.015</td>
<td>-0.280±0.023</td>
<td>0.016±0.001</td>
<td>0.128±0.005</td>
<td>0.037±0.002</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>60</td>
<td>-0.271±0.041</td>
<td>-0.280±0.031</td>
<td>0.025±0.002</td>
<td>0.180±0.005</td>
<td>0.053±0.002</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>72</td>
<td>-0.260±0.019</td>
<td>-0.273±0.012</td>
<td>0.033±0.002</td>
<td>0.216±0.014</td>
<td>0.064±0.002</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>96</td>
<td>-0.371±0.032</td>
<td>-0.381±0.023</td>
<td>0.018±0.001</td>
<td>0.150±0.004</td>
<td>0.052±0.003</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>60</td>
<td>-0.374±0.013</td>
<td>-0.382±0.014</td>
<td>0.023±0.003</td>
<td>0.167±0.018</td>
<td>0.059±0.004</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>96</td>
<td>-0.375±0.009</td>
<td>-0.386±0.012</td>
<td>0.032±0.003</td>
<td>0.204±0.012</td>
<td>0.074±0.003</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>48</td>
<td>-0.187±0.040</td>
<td>-0.213±0.036</td>
<td>0.018±0.001</td>
<td>0.137±0.003</td>
<td>0.036±0.002</td>
<td>0.013±0.002</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>70</td>
<td>-0.184±0.019</td>
<td>-0.200±0.015</td>
<td>0.029±0.002</td>
<td>0.187±0.010</td>
<td>0.052±0.002</td>
<td>0.013±0.003</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>48</td>
<td>-0.184±0.021</td>
<td>-0.213±0.025</td>
<td>0.031±0.003</td>
<td>0.196±0.020</td>
<td>0.056±0.007</td>
<td>0.020±0.002</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>72</td>
<td>-0.221±0.010</td>
<td>-0.235±0.010</td>
<td>0.017±0.002</td>
<td>0.134±0.010</td>
<td>0.038±0.003</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>70</td>
<td>-0.219±0.024</td>
<td>-0.229±0.020</td>
<td>0.027±0.001</td>
<td>0.185±0.004</td>
<td>0.051±0.002</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>72</td>
<td>-0.211±0.030</td>
<td>-0.234±0.020</td>
<td>0.034±0.001</td>
<td>0.197±0.018</td>
<td>0.058±0.003</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>96</td>
<td>-0.303±0.020</td>
<td>-0.316±0.012</td>
<td>0.015±0.002</td>
<td>0.130±0.004</td>
<td>0.041±0.001</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>70</td>
<td>-0.284±0.034</td>
<td>-0.304±0.033</td>
<td>0.020±0.002</td>
<td>0.140±0.018</td>
<td>0.046±0.006</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>96</td>
<td>-0.276±0.049</td>
<td>-0.303±0.036</td>
<td>0.031±0.002</td>
<td>0.198±0.005</td>
<td>0.064±0.002</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>
Table 4. F-ratio values and significance levels obtained in multifactor ANOVA for the physico-chemical parameters according to the factors: salt dose (S), relative humidity (RH), processing time (t) and their respective two-way interactions.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>S</th>
<th>RH</th>
<th>t</th>
<th>S x RH</th>
<th>S x t</th>
<th>RH x t</th>
</tr>
</thead>
<tbody>
<tr>
<td>X^w</td>
<td>10.19*</td>
<td>7.25*</td>
<td>111.85**</td>
<td>0.58ns</td>
<td>0.67ns</td>
<td>1.85ns</td>
</tr>
<tr>
<td>a_w</td>
<td>238.99***</td>
<td>33.22**</td>
<td>153.17***</td>
<td>2.44ns</td>
<td>2.75*</td>
<td>10.18***</td>
</tr>
<tr>
<td>X^NaCl</td>
<td>215.57***</td>
<td>35.67***</td>
<td>8.68***</td>
<td>2.31ns</td>
<td>5.81*</td>
<td>8.39*</td>
</tr>
<tr>
<td>X^NaCl</td>
<td>113.45***</td>
<td>11.73*</td>
<td>5.89**</td>
<td>1.96ns</td>
<td>5.70**</td>
<td>2.33ns</td>
</tr>
<tr>
<td>X^NaCl</td>
<td>228.18***</td>
<td>41.35***</td>
<td>22.94***</td>
<td>2.04ns</td>
<td>4.81**</td>
<td>11.22***</td>
</tr>
<tr>
<td>ΔM^o_t</td>
<td>1.02ns</td>
<td>76.09***</td>
<td>109.63***</td>
<td>0.12ns</td>
<td>3.76*</td>
<td>0.16ns</td>
</tr>
<tr>
<td>ΔM^w_t</td>
<td>1.44ns</td>
<td>9.03**</td>
<td>17.41***</td>
<td>2.93ns</td>
<td>0.20ns</td>
<td>7.12***</td>
</tr>
<tr>
<td>ΔM^NaCl_t</td>
<td>235.01***</td>
<td>0.63ns</td>
<td>8.90***</td>
<td>0.13ns</td>
<td>3.45*</td>
<td>1.71ns</td>
</tr>
<tr>
<td>Exudate (w/w)</td>
<td>0.86ns</td>
<td>12.99***</td>
<td>49.40***</td>
<td>0.68ns</td>
<td>0.86ns</td>
<td>12.68***</td>
</tr>
</tbody>
</table>

ns: no significant, *p <0.05, **p <0.01, ***p <0.001