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Feasibility of processing temperatures on the quality and shelf-life of smoke-flavoured cod

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ABSTRACT

The feasibility of two processing temperatures on the quality and shelf-life of smoke-flavoured cod was studied. Cod was submitted to a smoke-flavouring process in water vapour permeable (WP) bags at 5 or 10 °C. Physicochemical and microbiological analyses were run for 40 days of cold storage. The WP bags allowed the exudate to evaporate during the smoke-flavouring process, which enabled salting, drying and smoking to be done in a single step. Processing temperature did not bring about major changes in the moisture, pH, a_w and colour of the smoke-flavoured cod. However, processing at 10 °C increased volatile basic nitrogen (TVB-N) and trimethylamine nitrogen (TMA-N) from the start of the study, whereas the samples obtained at 5 °C maintained low TVB-N and TMA-N values, but only until day 21. The samples processed at 10 °C gave the highest Hx values due to degradation of IMP into Ino, and Ino into Hx. Microbiological counts were higher for the samples processed at 10 °C compared to samples processed at 5 °C, which did not reach the acceptability limits until day 40. Overall, the results provided of this study highlight the potential of smoke-flavouring process and in particular, the benefits of the use of refrigeration temperatures of 5 °C.

1. Introduction

Smoking has been applied since ancient times to extend the shelf-life of fish products. Traditionally, smoking process involves different stages: salting, drying and smoking. Fish preservation has been achieved by the synergetic action of salt uptake, the partial dehydration of the tissues that occurs throughout various process stages and the preservative action of smoke compounds (Goulas & Kontominas, 2005).

Largely, refrigeration combined with new packaging technologies has lessened the necessity for high contents of salt and smoke components to preserve fish (Birkeland, Rørå, Skåra, & Bjerkeng, 2004). In this context, smoke flavourings offer several advantages compared with traditional smoking, such as the possibility of avoiding harmful components for human health and environment, lower investments in acquiring the required equipment, and the fact that use, dose and handling are much easier, less expensive and less time-consuming (Muratore, Mazzaglia, Lanza, & Licciardello, 2007). For these reasons, the possibility of extending applications of smoke flavourings compared to conventional smoking has to be taken into account in the fish industry, where smoke-flavoured fish appears to be a good alternative to traditional smoked products. In line with this, Rizo, Máñes, Fuentes, Fernández-Segovia, and Barat (2015a) proposed a new process to obtain smoke-flavoured fish based on the application of water vapour permeable materials. This methodology consists of a simultaneous smoking-salting step, performed in water vapour permeable (WP) bags, under the established temperature and relative humidity conditions (RH) to facilitate product dehydration control. The use of smoke flavourings provides the typical smoked flavour to the product, and smoking and salting steps can be performed in a single stage. This procedure enabled us to obtain smoke-flavoured salmon with similar physicochemical characteristics and sensory acceptance to the smoked salmon product currently available on the market, with good hygienic quality, under cold storage (Rizo et al., 2015a; Rizo, Mañes, Fuentes, Fernández-Segovia, & Barat, 2015b). The use of this methodology could be an interesting alternative to traditional cold smoking of fish since the physicochemical properties, consumer acceptance and safety of the final product are not affected, it minimises product handling and brine waste, and cuts processing steps. This methodology has been tested in cod by adapting the processing parameters to the specific features of this fish species

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(Rizo, Fuentes, Fernández-Segovia, & Barat, 2014; McDonagh, 2014).

The quality and shelf-life of smoked products depend on raw material characteristics, hygienic practices during handling, the salting and smoking method, and processing conditions, such as the salt dose, duration and temperature of the process (Rørå et al., 2005). Processing temperature is critical as regards maintaining hygienic quality. The effects of smoking and drying temperature on fish quality have been studied (Goulas & Kontominas, 2005; Rørå et al., 2005), as well as the effect of salting temperature (Birkeland & Bjerkeng, 2005). In addition, refrigeration temperatures within the 3-12 °C range have been recommended for preventing microbial growth during salting. However, temperatures used for drying and cold smoking are usually between 16 and 30 °C (Codex, 2003). Although other researchers have evidenced processing temperature effects on quality of smoked fish accomplished by traditional techniques, how processing temperatures can affect the quality of smoke-flavoured fish products, in which salting and drying take place simultaneously, remains unknown. Therefore, the effect of two smoke-flavouring temperatures (5 and 10 °C) using WP bags on the quality and shelf-life of smoke-flavoured cod was studied.

2. Materials and methods

2.1. Materials

Fillets of frozen Atlantic cod (*Gadus morhua*) obtained from Alimentos Friorizados, S.A. (Barcelona, Spain), commercial size of 1.2–1.4 kg, were employed as raw material. Before processing, fillets (n = 10) were thawed at 4 °C for 24 h. Then cod fillets were trimmed to remove bones and cut into 4-cm portions to obtain approx. five portions per fillet (48 samples were obtained in all). The average weight of fish portions was 136 ± 23 g, and thickness was 2–3 cm. The initial microbial and physicochemical characterisation of the raw material was carried out.

The salt used for the smoke-flavouring process was supplied by Panreac Química, S.A. (Barcelona, Spain). Natural liquid smoke (HARDWOOD AFS 10, Amcan Ingrédients Ltd., Le Chesnay, France) consisting of a natural water-soluble condensate from the pyrolisis of walnut, maple, and other hardwoods, was applied to samples. Water vapour permeable (WP) bags were supplied by TUB-EX ApS (Taars, Denmark) (polyamide mix; size 200 × 300 × 0.04 mm; water vapour transmission rate 5,000 g/50μ/m^2/24 h (38°C/50% RH)).

2.2. Experimental design

Cod samples were subjected to a simultaneous smoke-flavouring procedure (Fig. 1) according to the method of Rizo et al. (2015a) to obtain smoke-flavoured fish. Liquid smoke, previously diluted in distilled water (60 mL/100 mL solution), was applied to surface of fish portions by spraying for 30 s. Portions were salted by dosing an amount of salt (2 g salt/100 g cod), according to previous studies (Rizo et al., 2014), to achieve similar a_w, salt and moisture content values to those of commercial smoked cod (Karásková et al., 2011; Rizo et al., 2014). Cod portions were vacuum-packed (Tecnotrip mod. EV-25-CD, Barcelona, Spain) in highly water vapour permeable bags. It should be noted that vacuum packing was used just to ensure initial contact between fish and the WP bag, since vacuum conditions cannot be maintained in these bags for a long time. Then cod samples were divided into two batches, which were processed at different temperatures (batch 1 at 5 °C and batch 2 at 10 °C). The smoke-flavouring of cod was carried out in a drying chamber (Binder mod. KBF. Tuttlingen, Germany) at 60% RH for 96 h.

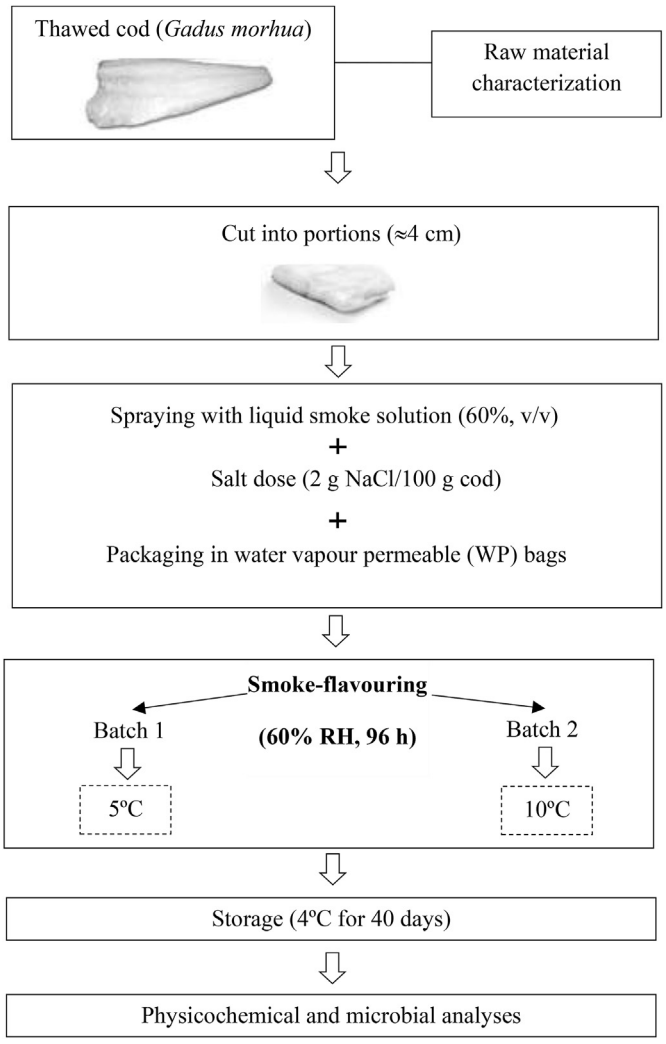

Fig. 1. Schematic representation of the smoke-flavouring process. RH: Relative Humidity.

At the end of the smoke-flavouring time, cod samples were removed from the bags. They were then placed in saturated brine with constant stirring for 30 s to remove any traces of salt attached to the surface, and were dried with absorbent paper and weighed. Finally, smoke-flavoured fillets were vacuum-packed in high barrier bags and stored at 4 °C for 40 days. These conditions were selected given its wide use in industry to store such products during their marketing period. The obtained smoke-flavoured cod was characterised by analyses of moisture, NaCl content, pH and a_w at day 0. Physicochemical and microbiological analyses of samples were performed at days 0, 7, 14, 21, 28, 35 and 40. Three samples were taken on each sampling day (n = 3). Analyses were performed on each sample in duplicate, except for pH, which was measured in quintuplicate.

2.3. Analytical determinations

2.3.1. Physicochemical analysis

Moisture contents (g/100 g) were determined in accordance with AOAC method 950.46 (1997). Sodium chloride content in the liquid phase (Z^{NaCl} (g NaCl/mL)) was measured in accordance with the procedure described by Fuentes, Fernández-Segovia, Serra, and

Barat (2010). pH measurements were taken with a micropH 2001 digital pH-meter (Crison Instruments, S.A., Barcelona, Spain) and a puncture electrode (Crison 5231) at five different sample locations. Water activity (a_w) was measured in minced samples with a fast water activity-meter (Aqualab dew point hygrometer model 4 TE, Decagon Devices, Inc., Washington, USA). Total volatile basic nitrogen (TVB-N) and trimethylamine nitrogen (TMA-N) contents (mg N/100 g) were determined according to the method described by Malle and Tao (1987). Weight changes in fish samples (ΔMt) were calculated according to Eq. (1).

$$\Delta M_t = \left(\frac{M_t - M_0}{M_0}\right) \quad (1)$$

where M_t is the sample weight at time t (g) and M_0 is the initial sample weight (g)

HPLC was used to determine the ATP-related compounds, consisting of inosine-50-monophosphate (IMP), inosine (Ino), and hypoxanthine (Hx), following the method described by Rizo et al. (2015b). K_1-value was calculated in accordance with Eq. (2):

$$K_1(\%) = \frac{[Ino] + [Hx]}{[IMP] + [Ino] + [Hx]} \times 100 \quad (2)$$

where IMP is inosine 5′-monophosphate, Ino is inosine and Hx is hypoxanthine (μmol/g).

2.3.2. Colour determination

Colour determination was performed on the surface of the cod fillets. A Minolta CM-700-d photocolorimeter (Minolta, Osaka, Japan) was used, with a 10° observer and illuminant D65. The sample was covered with low reflectance optical glass CR-A5/1829–752M to prevent any deterioration to the integrating sphere. Using the CIE L*a*b* coordinates (where L* is lightness, a* deviation towards red or green, and b* deviation towards yellow or blue), the psychophysical magnitudes of hue (h_{ab}*) and chroma (C_{ab}*) were calculated using Eqs. (3) and (4), respectively.

$$C_{ab}^* = \sqrt{a^{*2} + b^{*2}} \quad (3)$$

$$h_{ab}^* = arctg\,(b^*/a^*) \quad (4)$$

2.4. Microbiological analyses

Mesophilic bacteria and Enterobacteriaceae were determined according to the methods in Standard 4833:2003 and 21528–2:2004, respectively. The results were expressed as log cfu/g. All the culture media were purchased from Scharlau Chemie, S.A. (Barcelona, Spain).

2.5. Statistical analysis

One-way analysis of variance (ANOVA) was conducted to establish significant differences between the fresh samples and those recently smoked at 5 and 10 °C. Physicochemical and microbiological parameters were analysed by a multifactor ANOVA to evaluate the effect of processing temperature and storage time. The least significant difference (LSD) procedure was used to test for differences between averages at the 5% significance level. Statistical treatment was performed using Statgraphics Centurion XVI (Manugistics Inc., Rockville, MD, USA).

3. Results and discussion

3.1. Effect of the smoke-flavouring process

The physicochemical, microbiological and colour parameters analysed in the fresh cod used as raw material and in the recently smoke-flavoured cod are shown in Table 1. Compared with fresh cod, the smoke-flavouring process noticeably reduced water content, increased the NaCl concentration and lowered the a_w values probably due to NaCl uptake and dehydration.

The smoke-flavouring process led to a drop in pH, compared with the raw material values possibly due to the greater ionic strength of the internal solution in fish muscle cells associated with salt uptake (Leroi & Joffraud, 2000). The obtained smoke-flavoured product fulfilled the *Codex* standard for smoked fish, smoke-flavoured fish and smoked dried fish (*Codex,* 2013), which requires a minimum salt content of 5% ($z^{NaCl} = 0.05$) for smoke-flavoured fish, where smoke flavour is provided by artificial flavour blends in order to prevent the growth of *Clostridium botulinum* at storage temperatures of 3–10 °C. For both processing temperatures, the recorded moisture, a_w, pH and NaCl of smoke-flavoured product fell within the range of those reported elsewhere for commercially available smoked cod. Moreover, processing temperature did not significantly affect these parameters.

Total volatile basic nitrogen (TVB-N) and trimethylamine nitrogen (TMA-N) are widely used as indicators of fish spoilage (Okpala, Choo, & Dykes, 2014; Özoğul & Balikci, 2013). In fresh fish of good quality, total volatile basic nitrogen content is typically less than 20 mg N/100 g (Cardinal et al., 2004). Critical limits of 35 mg TVB-N/100 g have been established by the European Union for unprocessed fishery products species of *Gadidae* family such as cod (CEE, 2005), although these values can vary widely depending on whether the fish is fresh or processed (Siriskar, Khedkar, & Lior, 2013).

The TVB-N and TMA-N values of the raw material were 10.60 and 2.26 mg N/100 g of fish, respectively, which are in agreement with values for fresh cod reported by Ruiz-Rico et al. (2013), who found TVB-N levels ranging between 10 and 14 mg N/100 g. A significant increase in these parameters was observed after the smoke-flavouring process, which could be partially explained by the dehydration that the smoke-flavouring process causes, and the subsequent concentration of TVB-N and TMA-N, as observed in other studies (Goulas & Kontominas, 2005). However, this increase was significantly higher ($p < 0.05$) for the smoke-flavoured cod processed at 10 °C (39.38 mg N/100 g) as it almost doubled that recorded for the cod processed at 5 °C (20.95 mg N/100 g). These results suggest a higher level of spoilage in the smoke-flavoured cod at 10 °C, which correlated with the microbial growth described below.

Counts of mesophilic bacteria and Enterobacteriaceae in the samples that had been smoked at 5 °C appeared stable. However, when the processing temperature was 10 °C, higher levels of mesophilic bacteria and Enterobacteriaceae were found, which came close to the upper limits of acceptability (7 log cfu g^{-1} and 3 log cfu g^{-1}, respectively) set in other studies (Fuentes, Fernández-Segovia, Barat, & Serra, 2011; ICMSF, 1986).

The low values obtained for TVB-N, TMA-N, mesophilic bacteria and Enterobacteriaceae in fresh cod indicated that the raw material used in the present study exhibited good hygienic quality.

Regarding colour, the smoke-flavoured cod samples obtained lower L* values and higher a*and b* values compared with the fresh cod samples (Table 1). The reduction in lightness may be caused by the dehydration and protein denaturation that probably occurred in the fish during smoking processes, similarly to that reported by Birkeland et al. (2004). The increase in coordinates a* and b* can be

Table 1
Physicochemical, microbiological and colour parameters of fresh and smoke-flavoured cod at day 0. (Means and standard deviations, n = 3).

	Fresh cod	Smoke-flavoured cod		α
Processing temperature		5 °C	10 °C	
Moisture (g H_2O/100 g)	82.93 ± 0.90[a]	71.21 ± 1.41[b]	72.86 ± 1.87[b]	***
Z^{NaCl} (g NaCl/mL)	0.005 ± 0.001[a]	0.055 ± 0.003[b]	0.053 ± 0.005[b]	***
a_w	0.994 ± 0.001[a]	0.955 ± 0.009[b]	0.955 ± 0.004[b]	***
ΔM_t	–	−0.368 ± 0.034[a]	−0.345 ± 0.040[a]	ns
pH	6.76 ± 0.06[a]	6.32 ± 0.17[b]	6.54 ± 0.34[b]	**
TVB-N (mg N/100 g)	10.60 ± 4.27[a]	20.95 ± 2.42[b]	39.38 ± 9.09[c]	**
TMA-N (mg N/100 g)	2.26 ± 0.80[a]	7.19 ± 0.06[a]	25.53 ± 2.42[b]	***
Mesophilic bacteria (log cfu/g)	4.17 ± 0.27[a]	4.58 ± 0.25[b]	5.94 ± 0.13[c]	***
Enterobacteriaceae (log cfu/g)	1.45 ± 0.41[a]	1.54 ± 0.09[a]	3.00 ± 0.58[b]	**
IMP (μmol/g)	3.36 ± 0.03[a]	3.98 ± 0.88[a]	0.57 ± 0.19[b]	**
Ino (μmol/g)	1.87 ± 0.31[a]	2.05 ± 0.18[a]	1.92 ± 0.81[a]	ns
Hx (μmol/g)	0.24 ± 0.02[a]	0.94 ± 0.10[b]	3.41 ± 0.25[c]	***
K_1-value	38.42 ± 3.54[a]	49.04 ± 2.74[b]	89.33 ± 2.67[c]	***
L^*	56.55 ± 6.22[a]	49.88 ± 2.61[b]	48.87 ± 4.04[b]	*
a^*	−4.24 ± 0.30[a]	−2.14 ± 1.29[b]	−3.28 ± 0.93[ab]	*
b^*	−2.74 ± 2.24[a]	10.71 ± 1.44[b]	6.12 ± 2.39[b]	**
C_{ab}^*	5.44 ± 0.80[a]	11.02 ± 1.21[b]	7.44 ± 0.39[ab]	*
h_{ab}^*	0.66 ± 0.18[a]	178.85 ± 0.75[b]	179.07 ± 3.52[b]	***

z^{NaCl}: NaCl concentration in liquid phase; TVB-N: total volatile basic nitrogen; TMA-N: trimethylamine nitrogen; IMP: inosine-5′-monophosphate; Ino: inosine; Hx: hypoxanthine. Mean values ± SD (n = 3). Different letters indicate significant differences.
ns: no significant $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

associated with the application of liquid smoke to the fillets' surface, and also to the dehydration that causes yellowing on salted cod (Oliveira, Pedro, Nunes, Costa, & Vaz-Pires, 2012). Processing temperature had no significant effect on the colour of cod samples.

The inosine 5′-monophosphate, inosine and hypoxanthine values of the fresh cod and smoke-flavoured fish at day 0 are shown in Table 1. The raw material showed values for these parameters consistent with those found in another study of fresh cod (Ruiz-Rico et al., 2013). The smoke-flavouring process conducted at 5 °C did not significantly affect the IMP and Ino concentrations. However, when smoke-flavouring was conducted at 10 °C, the IMP value noticeably dropped, probably due to the higher spoilage at this temperature. The samples processed at 10 °C gave the highest Hx values due to degradation of IMP into Ino, and Ino into Hx. Likewise, the K_1-values were significantly affected by processing temperature. The initial K_1-value of samples processed at 5 °C was much lower than the value of the samples processed at 10 °C.

No exudate was collected from the bags after the process; the WP bags were permeable enough to allow the water released by fish muscle to largely evaporate, thus less brine waste would be generated. As previously highlighted herein, the salting, drying and smoking stages can be carried out in a single step with this process, which would reduce processing steps compared to traditional methods, where salting, drying and/or smoking are conducted separately. Furthermore, smoke-flavouring inside a bag enables fish processing to take place under more controlled conditions, which entails a lower risk of microbiological contamination during smoke-flavouring as bags of this type offer a barrier to bacteria (Rizo et al., 2015b).

3.2. Physicochemical quality evolution during storage

3.2.1. pH, TVB-N and TMA-N

The pH, TVB-N and TMA-N changes during the storage of smoke-flavoured cod are shown in Fig. 2 pH values remained constant throughout storage as only minor fluctuations were noted, which is in agreement with the results obtained by other authors (Goulas & Kontominas, 2005). This parameter was not affected by processing temperature during storage. This revealed that pH was not useful for monitoring fish quality changes, which is consistent with other studies (Arkoudelos, Stamatis, & Samaras, 2007; Li et al., 2011).

As previously mentioned, TVB-N is a common indicator of spoilage for many fish species. The limits of acceptability proposed by some authors for smoked fish fall within 30–40 mg N/100 g (Dalgaard, 2000). However, higher limits of acceptability have been given for salted and dried fish. Values higher than 75 mg N/100 g have been found in sugar-salted herring with acceptable sensory quality (Dalgaard, 2000), 50–110 mg N/100 g fish in dried-salted tuna products (Gallart-Jornet, Escriche, Chilet, & Fito, 2005) and 57 mg N/100 g in salted anchovies (Oetterer et al., 2003). Herein, TVB-N values of cod samples steadily increased with storage (Fig. 2). The samples obtained at 10 °C showed significantly higher TVB-N values compared to those processed at 5 °C during the first weeks of storage. From day 28, the values of this parameter were similar for both sample types.

The TMA-N values obtained in the smoke-flavoured cod samples displayed a similar pattern to TVB-N during storage. As with TVB-N, a wide variability in the limits of acceptability was seen for TMA-N. In general, 10–15 mg/100 g could be considered the upper limit for this parameter (Connell, 1995). Although TMA-N is believed to be generated by the action of spoilage bacteria, the correlation found with bacterial numbers is not often very good (Huss, 1995), consistent with the present study, as reported in other studies of smoked fish (Joffraud et al., 2006; Fuentes et al., 2011).

According to the limits of acceptability proposed for TVB-N and TMA-N, the smoke-flavoured cod processed at 10 °C showed spoilage from the beginning of the study. In contrast, the smoke-flavoured cod obtained at 5 °C did not exceed these values until storage day 21. Nevertheless, some studies have suggested that a single biochemical or microbiological parameter could not be used alone to precisely evaluate fish shelf-life (Leroi, Joffraud, Chevalier, & Cardinal, 2001; Jørgensen Dalgaard & Huss, 2000). For this reason, the TVB-N and TMA-N values should be compared with the other quality parameters.

3.2.2. ATP-related compounds and K_1-value

IMP, Ino and Hx contents, as well as the K_1-value, of the cod kept under cold storage of 40 days are shown in Fig. 3.

The breakdown of IMP into Ino, and Ino into Hx, is caused by endogenous enzymes. However, the hydrolysis of Ino and Hx formation may also result from the action of bacterial enzymes

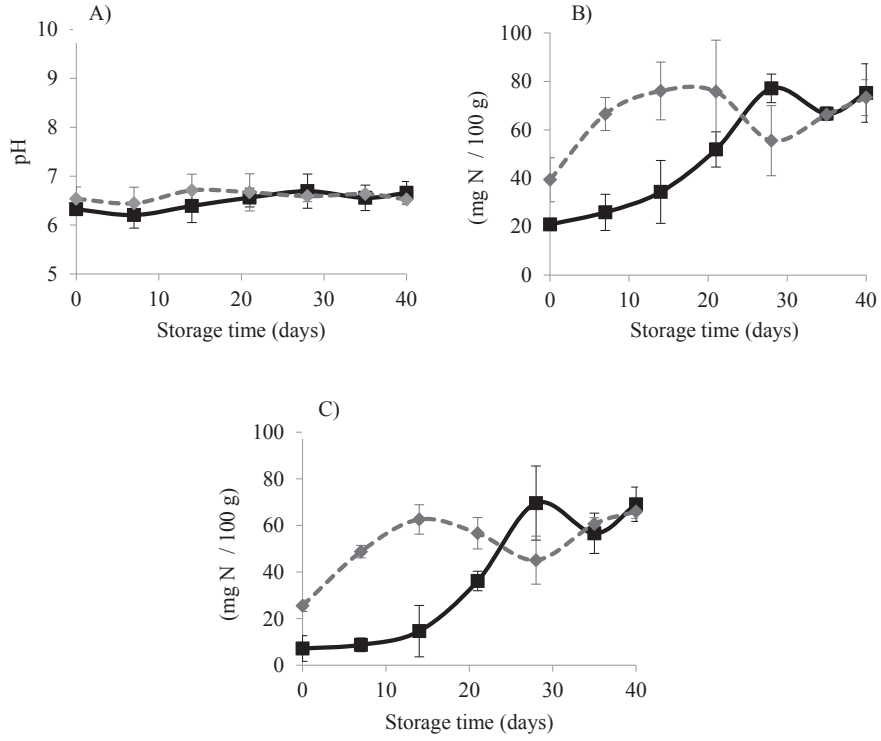

Fig. 2. Evolution of pH (A), TVB-N (B), and TMA-N (C) in smoke-flavoured cod samples processed at different temperatures (5°(■)) and 10 °C (◆)) during 40 days of storage at 4 °C. (Means and standard deviations, n = 3).

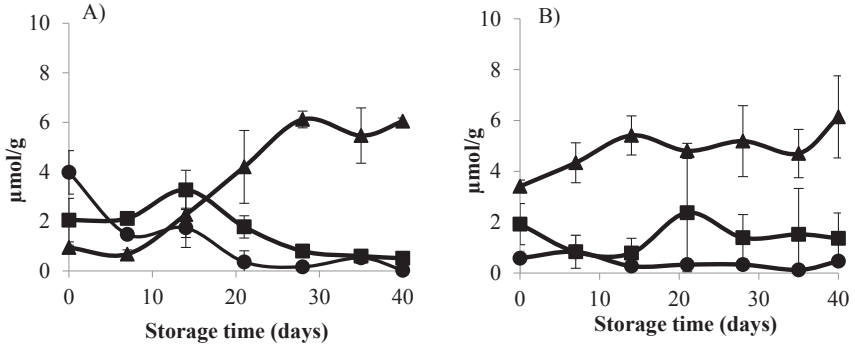

Fig. 3. Evolution of inosine-5′-monophosphate (IMP (●)), inosine (Ino (■) and hypoxanthine (Hx (▲)) in the smoke-flavoured cod samples processed at different temperatures (5 °C (A) and 10 °C (B)), during 40 days of storage at 4 °C. (Means and standard deviations, n = 3).

(Fernández-Segovia, Escriche, & Serra, 2008).

A progressive lowering of IMP contents, along with an increase in Hx contents, was observed for both sample types throughout storage. During the first weeks, higher IMP values and lower Hx values were obtained for the samples processed at 5 °C compared with those processed at 10 °C. However, similar values were found for both samples from day 21.

The K_1-value quantifies the extent of IMP degradation (Fig. 4). The initial K_1-values of the samples obtained at 5 °C were low (49%), but increased sharply to reach values over 93% by the end of the study. Higher values were recorded for the smoke-flavoured cod processed at 10 °C, which varied slightly during the study (from 89 to 98%) as IMP degradation occurred almost completely at the beginning of storage. These results correlate with the loss of freshness in cod observed by other authors (Fernández-Segovia et al., 2008; Ruiz-Rico et al., 2013).

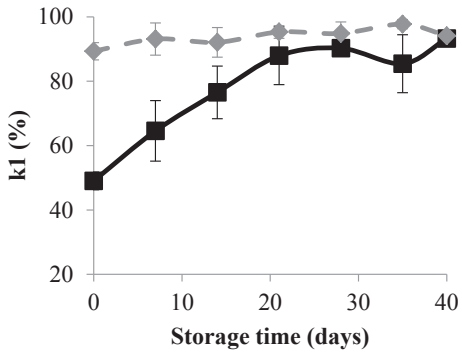

Fig. 4. Evolution of the K_1-value in the smoke-flavoured cod samples processed at different temperatures (5 °C (■) (A) and 10 °C (◆) (B)) during 40 days of storage at 4 °C. (Means and standard deviations, n = 3).

3.2.3. Colour parameters

The changes in the colour parameters of the smoke-flavoured cod during storage are shown in Table 2.

Neither processing temperature nor storage time had a significant effect on the colour parameters ($p > 0.05$). These results differ from those obtained in a previous work, in which significant colour differences were reported during storage in smoke-flavoured salmon obtained by the same method (Rizo et al., 2015b). In addition, different studies have highlighted the influence of the smoking temperature in the colour of smoked fish (Birkeland & Bjerkeng, 2005). However, it seems that smoke-flavouring temperature does not influence these parameters.

3.3. Microbiological analyses

The evolution of mesophilic and Enterobacteriaceae throughout the 40 days of storage is shown in Fig. 5.

During storage, mesophilic bacteria increased sharply for both processing temperatures. The samples obtained at 10 °C exhibited greater microbial growth for mesophilic bacteria, which exceeded the upper limit of acceptability (7 log cfu g-1) from storage day 7, whereas the samples obtained at 5 °C did not reach this limit during the study.

In cold smoked fish, the microbial flora present at the time of spoilage is variable and complex, with lactic acid bacteria and Enterobacteriaceae among the dominant microorganisms (Løvdal, 2015; Joffraud et al., 2006). In this sense, some studies have found high levels of mesophilic and lactic acid bacteria in cold smoked salmon (10^7-10^8 cfu g^{-1}) weeks before signs of spoilage became apparent, which sometimes make them unreliable quality indicators of cold smoked fish (Gram & Huss, 1996; Løvdal, 2015; Joffraud et al., 2006). In contrast, the presence of large numbers of Enterobacteriaceae is often associated with fish spoilage (Gram & Huss, 1996). Leroi et al. (2001) observed that shelf-life in smoked salmon was highly variable (1–6 weeks) and was related to the initial Enterobacteriaceae counts, which depends on the hygienic conditions during handling to a great extent. In this study, the initial Enterobacteriaceae counts were strongly affected by processing temperature. In the samples smoked at 10 °C, the above-cited limit of 3 log cfu g^{-1} was exceeded from the beginning of the study (day 0), but this limit was not reached until storage day 40 in the samples obtained at 5 °C. The lack of correlation between the values of the volatile bases (TVB-N and TMA-N) and the microbiological counts should be noted, especially for the cod processed at 5 °C.

According to the values obtained for TVB-N and TMA-N, the shelf-life of the smoke-flavoured fish processed at 5 °C would be shorter than 21 days. However, taking into account the results of Enterobacteriaceae the fish would not show spoilage until day 40. With the cod obtained at 10 °C, fish came close to the limit of acceptance from the beginning of the study, which indicates that the smoke-flavouring process at 10 °C was not adequate to obtain smoke-flavoured cod.

3.4. Statistical analysis

The multifactor ANOVA results obtained for each analysed parameter are shown in Table 3.

The analysed data revealed that storage time strongly influenced the TVB-N, TMA-N, IMP, Hx, K_1-value and mesophilic bacteria values. Processing temperature also showed significant effects on all the considered variables, with the exception of pH and Ino. In general, the interactions between storage time and processing temperature were less marked than when the factors were considered individually.

These results highlight the importance of conducting the smoke-flavouring process at refrigeration temperatures below 5 °C. The salting stage is especially critical in safety and quality terms as fish are more susceptible to spoilage when salt is not totally absorbed by muscle.

4. Conclusions

The water permeable (WP) bags allowed the exudate to completely evaporate during the smoke-flavouring process, which enabled salting, drying and smoking to be done in a single step. Processing temperature did not have effect on moisture, a_w, pH, colour or NaCl of the smoke-flavoured cod, but affected the initial values of TVB-N, TMA-N and microbiological counts. The smoke-flavoured cod recently obtained (day 0) at 10 °C came close to the microbiological and chemical limits of acceptance. In contrast, the smoke-flavoured cod processed at 5 °C exhibited an optimal initial microbiological quality. Only the smoke-flavouring process conducted at 5 °C enabled smoke-flavoured cod to be produced with an adequate degree of hygiene and with a shelf-life longer than 35 days in cold storage.

This methodology facilitates handling during chain production, and cuts processing steps and brine waste, which make the smoke-flavouring process simple and fast.

Table 2
Changes in colour parameters L* (lightness), a* (redness), b* (yellowness), C* (chroma), h* (hue) in the smoke-flavoured cod samples processed at different temperatures (5 and 10 °C) during 40 days of storage at 4 °C. (Means and standard deviations, n = 3).

t (days)	T (°C)	L*	a*	b*	C_{ab}*	h_{ab}*
0	5	49.88 ± 2.61^{aA}	-2.14 ± 1.29^{aA}	10.71 ± 1.44^{aA}	11.03 ± 1.20^{aA}	178.85 ± 0.75^{abA}
	10	48.87 ± 4.04^{aA}	-3.28 ± 0.93^{aA}	6.12 ± 4.39^{aA}	7.44 ± 3.52^{aA}	179.07 ± 0.39^{abA}
7	5	53.40 ± 3.09^{aA}	-2.60 ± 0.53^{aA}	10.83 ± 1.65^{abA}	11.17 ± 1.51^{abcA}	178.67 ± 0.07^{aA}
	10	50.62 ± 3.39^{aA}	-2.83 ± 1.28^{aA}	10.74 ± 4.12^{abA}	11.34 ± 3.59^{abcA}	178.75 ± 0.24^{aA}
14	5	52.97 ± 1.88^{aA}	-1.38 ± 1.16^{aA}	12.60 ± 2.89^{abA}	12.78 ± 2.61^{abcA}	178.57 ± 0.158^{aA}
	10	50.31 ± 5.33^{aA}	-2.93 ± 0.85^{aA}	7.79 ± 3.41^{abA}	8.67 ± 2.47^{abcA}	178.88 ± 0.39^{aA}
21	5	50.33 ± 2.34^{aA}	-3.02 ± 0.91^{aA}	8.96 ± 2.11^{abA}	9.55 ± 1.86^{abA}	178.77 ± 0.14^{aA}
	10	49.16 ± 4.93^{aA}	-1.51 ± 0.92^{aA}	11.13 ± 2.80^{abA}	11.27 ± 2.78^{abA}	178.57 ± 0.09^{aA}
28	5	49.14 ± 2.43^{aA}	-2.23 ± 0.76^{aA}	11.80 ± 1.71^{bA}	12.05 ± 1.60^{bcA}	178.83 ± 0.76^{abA}
	10	52.62 ± 4.59^{aA}	-1.77 ± 1.59^{aA}	12.71 ± 4.01^{bA}	12.99 ± 3.80^{bcA}	178.82 ± 0.76^{abA}
35	5	50.66 ± 2.14^{aA}	-1.00 ± 0.76^{aA}	12.73 ± 2.28^{bA}	12.80 ± 2.25^{cA}	178.83 ± 0.96^{aA}
	10	53.42 ± 2.64^{aA}	-3.12 ± 1.38^{aA}	12.13 ± 3.01^{bA}	12.65 ± 2.69^{cA}	178.70 ± 0.15^{aA}
40	5	48.32 ± 2.39^{aA}	-2.18 ± 1.17^{aA}	11.97 ± 1.31^{bA}	12.24 ± 1.17^{bcA}	178.84 ± 0.7^{bA}
	10	53.38 ± 4.32^{aA}	-1.73 ± 2.36^{aA}	11.97 ± 1.31^{bA}	12.92 ± 4.84^{bcA}	179.31 ± 0.47^{bA}

Different lower-case letters indicate significant differences for processing temperature factor (T).
Different capital letters indicate significant differences for processing temperature (t). ($p < 0.05$).

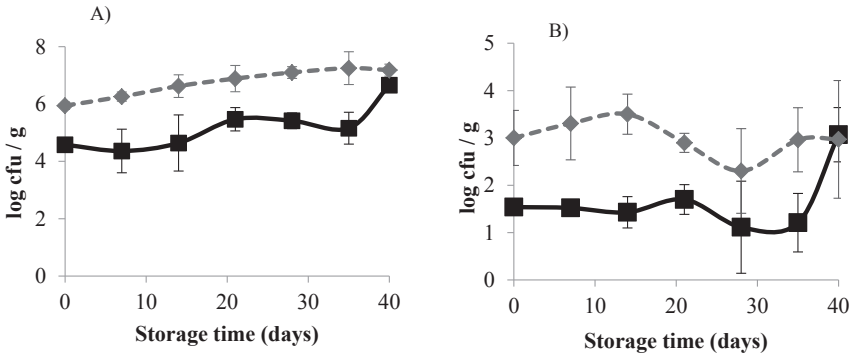

Fig. 5. Changes in mesophilic bacteria (A) and Enterobacteriaceae (B) in the smoke-flavoured cod samples processed at different temperatures (5 °C (■) (A) and 10 °C (◆) (B)) for 40 storage days at 4 °C. (Means and standard deviations, n = 3). Different letters indicate significant differences.

Table 3
F-ratio values and significance levels obtained in the multifactor ANOVA for the physicochemical and microbiological parameters according to factors storage time (t) and processing temperature, (T) and their interaction (t × T).

	t	T	t × T
pH	2.25^{ns}	1.26^{ns}	1.73^{ns}
TVB-N	12.68^{***}	14.12^{***}	7.50^{***}
TMA-N	29.05^{***}	32.26^{***}	11.26^{***}
IMP	13.85^{***}	29.94^{***}	9.12^{***}
Ino	1.96^{ns}	0.03^{ns}	2.97^{*}
Hx	13.91^{***}	7.29^{*}	7.04^{***}
K_1-value	26.17^{***}	104.41^{***}	15.16^{***}
Mesophilic bacteria	9.40^{***}	87.30^{***}	2.37^{ns}
Enterobacteriaceae	2.21^{ns}	40.98^{***}	1.75^{ns}

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

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References

AOAC. (1997). *Official methods of analysis* (16th ed.). Washington: Association of Official Analytical Chemists.
Arkoudelos, J., Stamatis, N., & Samaras, F. (2007). Quality attributes of farmed eel (*Anguilla anguilla*) stored under air, vacuum and modified atmosphere packaging at 0°C. *Food Microbiology, 24*, 728–735.
Birkeland, S., & Bjerkeng, B. (2005). The quality of cold-smoked Atlantic salmon (*Salmo salar*) as affected by salting method, time and temperature. *International Journal of Food Science and Technology, 40*(9), 963–976.
Birkeland, S., Rørå, M. B., Skåra, T., & Bjerkeng, B. (2004). Effects of cold smoking procedures and raw material characteristics on product yield and quality parameters of cold smoked Atlantic salmon (*Salmo salar L.*) fillets. *Food Research International, 37*, 273–286.
Cardinal, M., Gunnlaugsdottir, H., Bjoernevik, M., Ouisse, A., Vallet, J. L., & Leroi, F. (2004). Sensory characteristics of cold-smoked Atlantic salmon (*Salmo salar*) from European market and relationships with chemical, physical and microbiological measurements. *Food Research International, 37*(2), 181–193.
CEE. (2005). Commission regulation nº 2074/2005 of 5 December 2005, implementing measures for certain products under regulation (...). Section II, Chapter I: total volatile basic nitrogen (TVB-N) limit values for certain categories of fishery products and analysis methods to be used. *Official Journal of the European Union, L338*, 1.
Codex Alimentarius. (2003). *Code of practice for fish and fishery products. CAC/RCP 52*. Rome: FAO/WHO.
Codex Alimentarius. (2013). *Standard for smoked fish, smoke-flavoured fish and smoke-dried fish (311-2013)*. Rome: FAO/WHO.
Connell, J. J. (1995). *Control of fish quality* (4th ed.). London: Fishing News Books Limited.
Dalgaard, P. (2000). *Freshness, quality and safety in seafood. Flair-flow Europe technical manual F-FE 380A/00* Accessed 24.6.15 http://flairflow4.vscht.cz/seafood00.pdf.
Fernández-Segovia, I., Escriche, I., & Serra, J. A. (2008). Evolution of volatile fraction and ATP related compounds during storage of desalted cod *(Gadus morhua). Food Science and Technology International, 14*(5), 37–47.
Fuentes, A., Fernández-Segovia, I., Barat, J. M., & Serra, J. A. (2011). Influence of sodium replacement and packaging on quality and shelf-life of smoked sea bass (*Dicentrarchus labrax* L.). *LWT – Food Science and Technology, 44*(4), 917–923.
Fuentes, A., Fernández-Segovia, I., Serra, J. A., & Barat, J. M. (2010). Development of a smoked sea bass product with partial sodium replacement. *LWT - Food Science and Technology, 43*(9), 1426–1433.
Gallart-Jornet, L., Escriche, I., Chilet, A., & Fito, P. (2005). Estudio composicional y de parámetros de estabilidad de productos seco-salados derivados del atún. *Alimentación, Equipos y Tecnología. Equipos y Procesos para la Industria Alimentaria, 202*, 75–81.
Goulas, A. E., & Kontominas, M. G. (2005). Effect of salting and smoking-method on the keeping quality of chub mackerel (*Scomber japonicus*): biochemical and sensory attributes. *Food Chemistry, 93*(3), 511–520.
Gram, L., & Huss, H. H. (1996). Microbiological spoilage of fish and fish products. *International Journal of Food Microbiology, 33*(1), 121–137.
Huss, H. H. (1995). Quality and quality changes in fresh fish. In *Fisheries technical paper, No. 348*. Rome: FAO.
ICMSF. (1986). International commission on microbiological specifications for foods. Sampling plans for fish and shellfish. In ICMSF (Ed.), *Microorganisms in foods. Sampling for microbiological analysis: Principles and scientific applications* (2nd ed., Vol. 2, pp. 181–196). Toronto, Canada: University of Toronto Press.
Joffraud, J. J., Cardinal, M., Cornet, J., Chasles, J. S., Léon, S., Gigout, F., et al. (2006). Effect of bacterial interactions on the spoilage of cold-smoked salmon. *International Journal of Food Microbiology, 112*(1), 51–61.
Jørgensen, L. V., Dalgaard, P., & Huss, H. H. (2000). Multiple compound quality index for cold-smoked salmon (*Salmo salar*) developed by multivariate regression of biogenic amines and pH. *Journal of Agricultural and Food Chemistry, 48*(6), 2448–2453.
Karásková, P., Fuentes, A., Fernández-Segovia, I., Alcañiz, M., Masot, R., & Barat, J. M. (2011). Development of a low-cost non-destructive system for measuring moisture and salt content in smoked fish products. *Procedia Food Science, 1*, 1195–1201.
Leroi, F., & Joffraud, J. J. (2000). Salt and smoke simultaneously affect chemical and sensory quality of cold-smoked salmon during 5 °C storage predicted using factorial design. *Journal of Food Protection, 63*(9), 1222–1227.
Leroi, F., Joffraud, J. J., Chevalier, F., & Cardinal, M. (2001). Research of quality indices for cold-smoked salmon using a stepwise multiple regression of microbiological counts and physico-chemical parameters. *Journal of applied microbiology, 90*(4), 578–587.
Li, X., Li, J., Zhu, J., Wang, Y., Fu, L., & Xuan, W. (2011). Postmortem changes in yellow grouper (*Epinephelus awoara*) fillets stored under vacuum packaging at 0 °C. *Food Chemistry, 126*, 896–901.
Løvdal, T. (2015). The microbiology of cold smoked salmon. *Food Control, 54*, 360–373.
Malle, P., & Tao, S. H. (1987). Rapid quantitative determination of trimethylamine using steam distillation. *Journal of Food Protection, 50*(9), 756–760.
McDonagh, G. (2014). *Cold-smoked cod obtained using water vapour permeable bags*. Dissertation of Food Innovation Degree. Dublin Institute of Technology.
Muratore, G., Mazzaglia, A., Lanza, C. M., & Licciardello, F. (2007). Effect of process variables on the quality of swordfish fillets flavored with smoke condensate. *Journal Food Processing and Preservation, 31*, 167–177.
Oetterer, M., Perujo, S. D., Gallo, C. R., Arruda, L. F., Borghesi, R., & Cruz, A. M. P. (2003). Monitoring the sardine (*Sardinella brasiliensis*) fermentation process to obtain anchovies. *Scientia Agricola, 60*(3), 511–517.
Okpala, C. O. R., Choo, W. S., & Dykes, G. A. (2014). Quality and shelf life assessment of Pacific white shrimp (*Litopenaeus vannamei*) freshly harvested and stored on ice. *LWT – Food Science and Technology, 55*(1), 110–116.

Oliveira, H., Pedro, S., Nunes, M. L., Costa, R., & Vaz-Pires, P. (2012). Processing of salted cod (*Gadus spp.*): a review. *Comprehensive Reviews in Food Science and Food Safety, 11*(6), 546–564.

Özoğul, Y., & Balikci, E. (2013). Effect of various processing methods on quality of mackerel (*Scomber scombrus*). *Food and Bioprocess Technology, 6*(4), 1091–1098.

Rizo, A., Fuentes, A., Fernández-Segovia, I., & Barat, J. M. (2014). A novel process for obtaining a smoked cod product. In *44th WEFTA (West european fish technologists association) meeting – seafood science for a changing demand* ((Poster) Bilbao, Spain).

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

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

Rørå, A. M. B., Birkeland, S., Hultmann, L., Rustad, T., Skåra, T., & Bjerkeng, B. (2005). Quality characteristics of farmed Atlantic salmon (*Salmo salar*) fed diets high in soybean or fish oil as affected by cold-smoking temperature. *LWT - Food Science and Technology, 38*(3), 201–211.

Ruiz-Rico, M., Fuentes, A., Masot, R., Alcañiz, M., Fernández-Segovia, I., & Barat, J. M. (2013). Use of the voltammetric tongue in fresh cod (*Gadus morhua*) quality assessment. *Innovative Food Science & Emerging Technologies, 18*, 256–263.

Siriskar, D. A., Khedkar, G. D., & Lior, D. (2013). Production of salted and pressed anchovies (*Stolephorus* sp.) and its quality evaluation during storage. *Journal of Food Science and Technology, 50*(6), 1172–1178.