Development of a novel smoke-flavoured salmon product by sodium replacement using water vapour permeable bags

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Run title: Novel smoke-flavoured salmon product with reduced sodium content

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Abstract

BACKGROUND: Food manufacturers need to reduce sodium contents to meet consumer and public health demands. In this study the use of sodium-free (SF) salt and KCl to develop a novel smoke-flavoured salmon product with reduced sodium content was evaluated. Fifty percent of NaCl was replaced with 50% of SF salt or 50% KCl in the salmon smoke-flavouring process carried out using water vapour permeable bags.

RESULTS: Triangle tests showed that samples with either SF salt or KCl were statistically similar to the control samples (100% NaCl). Since no sensorial advantage in using SF salt was found compared with KCl and given the lower price of KCl, the KCl-NaCl samples were selected for the next phase. The changes of physicochemical and microbial parameters in smoke-flavoured salmon during 42 days showed that partial replacement of NaCl with KCl did not significantly affect the quality and shelf-life of smoke-flavoured salmon, which was over 42 days.

CONCLUSION: Smoke-flavoured salmon with 37% sodium reduction was developed without affecting sensory features and shelf-life. This is an interesting option for reducing sodium content in such products to help meet the needs set by both health authorities and consumers.

Keywords: Smoke-flavouring; salmon; sodium reduction; KCl; water vapour permeable bags; shelf-life.

INTRODUCTION

Smoking has been applied since ancient times to preserve fish. The shelf life of fish is prolonged by means of salt uptake, dehydration, and smoke absorption that fish undergo in the different smoking process stages (salting, drying and smoking).

In particular, salt plays a vital role to protect fish from spoilage in cold-smoked products. However, high salt levels go against current trends to reduce the amount of sodium in processed food in line with public health demands. Indeed, WHO Member States have agreed on a voluntary global target to achieve a dietary salt reduction in the mean population by 30%, which involves a salt intake lower than 5 g per day (approx. 2 g of sodium) by 2025.[1] Likewise, the EU has established a common framework to reduce salt intake in the general population.[2] The European approach towards reducing salt focuses on a limited number of food categories, which include fish products. To achieve this goal, the European Commission supports research and work programmes with industry on sodium reduction in foods up to the lowest possible level, while maintaining food safety and consumer acceptance. For this reason, some manufacturers are reformulating recipes to reduce the salt content of their products, and many studies focus on salt reduction and salt replacement in different food types.[3,4]

To date, the partial substitution of NaCl for KCl seems the best alternative to reduce sodium content as potassium intake has not been linked to the development of hypertension and cardiovascular diseases. KCl has similar properties as NaCl and can be used to produce low-sodium food products without affecting their functionality. Furthermore, KCl is listed as being generally recognised as safe (GRAS) and appears to have a similar antimicrobial effect as NaCl against foodborne pathogens,[5] such as *Listeria monocytogenes* [6] and *Clostridium botulinum* type E.[7] The problem with potassium is that it can impart bitter and metallic flavour to foods when used at high levels.[4] To overcome this limitation, application of flavour enhancers and bitter inhibitors to increase the saltiness perception, and to mask the aftertaste associated with potassium, are being studied.[8] In recent years, commercial salt substitutes have been introduced into the market to fully or partially replace sodium chloride in various products.[9] Many are KCl-based salt formulations, which contain flavour enhancers such as glutamic acid derivatives, like monosodium glutamate (MSG), often combined with 5´-ribonucleotides (disodium guanylate and disodium inosinate), as well as bitter inhibitors like amino acids lysine and taurine. The development of reduced-sodium fish products that do not affect product quality and safety is relevant as many governments attempt to promote fish consumption for its good nutritional characteristics. However, sodium replacement in fish products has not yet been extensively researched, and very few studies on salted cod, surimi, smoked sea bass and smoked salmon are available.[10-13]

The aim of this work was to assess the use of KCl and a KCl-based salt substitute to develop a reduced sodium smoke-flavoured salmon product with similar sensory features and shelf-life to smoke-flavoured salmon salted with 100% NaCl.

MATERIALS AND METHODS

Materials

The fish employed as raw material was aquacultured salmon (*Salmo salar*) from Norway (Marine Harvest, Bergen, Norway). It was purchased from a local market in the city of Valencia (Spain) and its commercial size was 2-3 kg. Salmons were headed, gutted and filleted, and two fillets per fish were obtained. Before processing, fillets were trimmed to remove bones and cut into 4-cm portions, which provided five portions per fillet. The average fish portion weight was 136±23 g and thickness was 2-3 cm.

In this study, a KCl-based salt substitute and two salt types (KCl and NaCl) were employed for salmon smoke-flavouring. The salt substitute was commercial sodium-free (SF) salt from the company Navarro e Hijos, S.A. (Alicante, Spain). Its composition included potassium chloride, l-lysine monohydrochloride, glutamic acid, potassium tartrate and silicon dioxide. NaCl and KCl salts were supplied by Panreac Química, S.A. (Barcelona, Spain). Natural liquid smoke (HARDWOOD AFS 10) was provided by Amcan Ingrédients Ltd. (Le Chesnay, France) and consisted in a natural water-soluble condensate from the pyrolisis of walnut, maple, and other hardwoods. The water vapour permeable (WP) bags used for smoke-flavouring were supplied by TUB-EX ApS (Taars, Denmark) (polyamide mix; size: 200×300×0.04 mm; water vapour transmission rate: 5,000 g/50μ/m²/24 h (38°C/50% RH).

Experimental design

Phase I: Evaluation of partial NaCl replacement with SF salt and KCl in smoke-flavoured salmon.

Salmon portions were subjected to a simultaneous smoking-salting procedure in which water vapour permeable (WP) bags were used following the method developed by Rizo et al.[14] to obtain smoke-flavoured fish (Fig. 1). Liquid smoke, previously diluted in distilled water (600 mL L^{-1} solution), was applied to the fish portions by spraying the fish surface for 30 s. Samples were randomly divided into three groups to be salted: the first with 100% NaCl (batch I); the second with the formulation that contained SF salt and NaCl at 50% (w/w) (batch II); the third with the formulation III that contained KCl and NaCl at 50% (w/w) (batch III). The NaCl substitution percentage was selected according to the results obtained in a previous work done in our laboratory, which concluded that NaCl could be replaced with up to 50% of SF salt without affecting the sensory and physicochemical traits of smoke-flavoured salmon. Portions were salted by means of a controlled process, dosing an amount of salt (70 g kg^{-1} fresh salmon) to achieve similar a_w, chloride content and moisture values to those of commercial smoked salmon.[14] Salt content was expressed as the chloride concentration in the liquid phase (z^{Cl-}) to properly compare all the experimental data, regardless of whether samples contained NaCl or mixtures of NaCl and SF salt or KCl.

Then, the salmon portions were vacuum-packaged (Tecnotrip mod. EV-25-CD, Barcelona, Spain) in highly water vapour permeable bags (WP). It should be noted that vacuum packaging was used just to ensure the initial contact between fish and the WP bag since vacuum conditions cannot be maintained for a long periods of time. The smoke-flavouring process was carried out in a drying chamber (Binder mod. KBF. Tuttlingen, Germany) for 24 h at 60% RH. When the processing time ended, salmon samples were removed from bags. They were then placed in saturated brine with constant stirring for 30 s to remove any traces of salt attached to the surface, dried with absorbent paper and weighed. The obtained raw material and smoke-flavoured salmon were characterised by analyses of moisture, chloride, sodium and potassium contents, a_w, and weight loss (ΔM_t). Moreover, sensory evaluations were carried out.

Phase II: Quality and shelf-life assessment of the smoke-flavoured salmon product

The objective of this second phase was to evaluate the quality and shelf-life of the smoke-flavoured salmon salted with 50% KCl-50%NaCl (selected in Phase I) during cold storage. Control samples were also prepared using 100% NaCl. The smoke-flavoured salmon samples, obtained according to the process illustrated in Fig. 1, were vacuum-packaged and stored for 42 days at 4ºC. The packaging and storage conditions were selected as being the most commonly used in industry for such products during their marketing period. Physicochemical and microbiological analyses were performed on the raw material on day 0 and on the smoke-flavoured product obtained on cold storage days 0, 7, 14, 21, 28, 35 and 42. Three samples of each salt type were taken (n=3) on each sampling day. Analyses were performed in duplicate on each sample, except for pH, which was measured in quintuplicate.

Sensory analyses

Two triangle tests[15] were carried out to test for similarity between the smoke-flavoured samples with NaCl replacement and the control samples. Sensory evaluations were made during two sessions with 66 untrained panellists. The first test was conducted with the samples salted by the 50%SF-50%NaCl formulation and the control samples (100% NaCl). During the second session, the smoke-flavoured salmon salted with 50% KCl-50% NaCl and the control samples were evaluated.

All the samples were obtained by the previously described smoke-flavouring procedure. Then they were filleted, vacuum-packaged and kept at 4ºC until the sensory evaluation was made (approx. 24 h after the whole process finished).

Assessors received a set of three samples and were informed that two of the samples were alike and one was different. Assessors were asked to report which sample they believed was different, even if the selection was based only on guesswork. Samples were randomly served on the same dish at room temperature and coded with a 3-digit random number.

Analytical determinations

Physicochemical analyses

Moisture content was determined by oven drying until constant weight at 105°C in accordance with AOAC method 950.46.[16] The lipid content of samples was determined by Soxhlet extraction using petroleum ether in accordance with AOAC method 991.36.[16]

Chloride content was determined in accordance with the procedure described by Fuentes et al.[12] after sample homogenisation in distilled water using an automatic Sherwood Chloride Analyser, Model 926 (Sherwood Scientific Ltd., Cambridge, UK). The same extract was used to analyse sodium. Potassium was analysed by absorption spectrophotometry in a Perkin-Elmer spectrophotometer model 3100 (Norwalk, CT, USA).

pH measurements were taken with a micropH 2001 digital pH-meter (Crison Instruments, S.A., Barcelona, Spain) with 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 4TE, Decagon Devices, Inc., Washington, USA).

Total volatile basic nitrogen (TVB-N) and trimethylamine nitrogen (TMA-N) contents were determined by steam distillation, following the method described by Malle and Tao.[17] The thiobarbituric acid (TBA) index was measured by a spectrophotometric method with minor modifications to evaluate oxidation stability during chilled storage.[18]

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, which consisted in inosine-5′-monophosphate (IMP), inosine (Ino), and hypoxanthine (Hx), following the method described by Rizo et al.[19] The K_1-values were calculated by Eq. 2.

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

where IMP is inosine 5′-monophosphate, Ino is inosine and Hx is hypoxanthine.

The shear force test was performed with a Texture Analyser TA.XT2® (Stable Micro Systems, Surrey, UK), equipped with an HDP/BS Warner-Bratzler test cell, which sliced samples perpendicularly to muscle orientation at a constant speed of 1 mm/s with a 90º angle-inverted knife. Samples were obtained by cutting out parallelepiped pieces (3 × 2 cm) from the same fish part. Shear force was determined by the maximum recorded force (N).

Microbiological analyses

Mesophilic bacteria and *Enterobacteriaceae* were determined according to the methods provided by ISO standard 4833:2003 and 21528-2:2004, respectively. [20,21] All the analyses were performed in duplicate and the results were expressed as log cfu/g. All the culture media were provided by Scharlau Chemie, S.A. (Barcelona, Spain).

Statistical analyses

A one-way ANOVA was conducted with the data of the physicochemical and microbial analyses of Phases I and II to test whether there were significant differences between the fresh and recently smoke-flavoured salmons obtained with the different salt formulations. The triangle test results were analysed using the corresponding table of triangle tests for similarity according to UNE-EN ISO 4120:2008.[15]

During the storage study, the data on each parameter were analysed by a multifactor ANOVA to evaluate the effect of salt formulation, storage time and their interactions. All the physicochemical and microbiological parameters were considered dependent variables. Salt formulation and storage time were taken as factors in these analyses. The least significant difference procedure was used to test for differences between averages at the 5% significance level. Data are reported as mean ± standard deviation. Statistical data processing was performed with the Statgraphics Centurion software (Statpoint Technologies, Inc., Warrenton, VA, USA).

RESULTS AND DISCUSSION

Phase I: Evaluation of partial NaCl replacement with SF salt and KCl in smoke-flavoured salmon.

Physicochemical analyses

Table 1 provides the results from the physicochemical characterisation of the smoke-flavoured samples obtained with 100% NaCl and by partial NaCl replacement.

The smoke-flavouring process led to a significant reduction in moisture and an increase in chloride content, which reduced a_w compared with the raw material. These changes were due to dehydration and salt absorption in muscle, which were related directly to shelf-life and sensory characteristics in smoked fish. The moisture, chloride content and

a_w of the different smoke-flavoured salmon samples were similar to those found for commercially available smoked salmon.[14,22] According to the *Codex* standard,[23] smoke-flavoured fish requires a minimum NaCl content of 50 g kg^{-1} (z^{NaCl} = 0.05) to prevent *Clostridium botulinum* from growing at storage temperatures between 3-10ºC, when the smoke flavour is provided by artificial flavour blends. To fulfil the *Codex* standard, the minimum chloride content value in this study should be at least 30 g kg^{-1} ($z^{Cl^-} \geq 0.03$) considering that NaCl is made up of 60% Cl^-. By taking into account these data, the products obtained with the different salt formulations fulfilled this requirement (Table 1).

The antimicrobial effect of KCl, compared to NaCl, with a view to NaCl replacement in food products has been confirmed by different studies.[5,6] These studies have reported that NaCl and KCl perform the same action against foodborne pathogens when present in food in equimolar KCl:NaCl mixtures, or when its concentration leads to an equivalent a_w in the product. In line with this, Pelroy et al.[7] demonstrated that it is feasible to substitute 50% NaCl for KCl in hot smoked fish without it affecting the inhibition of the *Clostridium botulinum* type E toxin formation. Thus, KCl is a safe alternative for reducing NaCl in smoked fish products. Partial NaCl replacement with SF salt and KCl significantly reduced the sodium content and increased the potassium content of smoke-flavoured salmon. The moisture and a_w values were similar in the three sample types and no significant differences among them were found. Weight loss was also similar in all these samples.

Sensory analyses

Two triangle tests for similarity were carried out to test if the samples salted with 50% SF-50% NaCl or 50% KCl-50%NaCl were perceived as being similar to the control samples (100% NaCl).

The first triangle test results revealed that no meaningful differences were perceptible between the samples salted with SF-NaCl and the control samples as the assessors correctly identified the odd sample in 22 cases of 66, unlike the tabulated value at $p<0.05$, which corresponded to 28.[15] Similar results were obtained in the second triangle test carried out with the KCl-NaCl and control samples, in which the panel correctly identified the different sample in 24 cases. These results show that there is no advantage in using SF salt to replace NaCl to produce smoke-flavoured salmon compared with employing KCl. It also appeared that smoke-flavoured salmon was potentially not as sensitive to NaCl replacement with KCl as other food types, probably because of the smoky flavour.[9,24] By taking into account the lower price of KCl compared with SF, and that sodium-free salt ingredients (l-lysine monohydrochloride and glutamic acid) do not offer any advantage in terms of masking the potassium aftertaste, the KCl-NaCl samples were selected for the next study phase.

Different results relating these ingredients have been reported in other studies. Dos Santos et al.[8] observed that using mixtures of KCl, lysine and monosodium glutamate sufficed to remove the defects caused by a 60-75% replacement of NaCl with KCl without affecting quality in fermented cooked sausages. Mitchell et al.[24] replaced 60% of NaCl in chilli con carne ready meals with a commercial mixture of KCl and L-lysine, and found no sensory differences in salty taste between the control and the low-salt samples that contained this salt substitute, nor any bitter or metallic flavours. However, when salting is applied to products whose initial structure must be preserved, such as smoked fish, salts and other ingredients must diffuse from the point of entry to the whole product, which means that transport by diffusion plays a vital role in the process.[25] The SF salt used herein was intended to be used as table salt. Hence the optimisation of the physical form of its ingredients to allow them to better penetrate inside the muscle and to interact with each other, may improve the masking effect of lysine and glutamic acid on potassium-bitterness, when KCl is used at high levels.

Phase II: Quality and shelf-life assessment of the smoke-flavoured salmon product

Effect of the smoke-flavouring process

The results of the parameters analysed in the fresh salmon used as raw material and in the recently smoke-flavoured salmon are shown in Table 2.

As expected, the smoking-salting process reduced water content and the a_w values due to salt uptake and dehydration. The moisture, a_w and chloride content values of the obtained smoke-flavoured products fell within the range of values reported for commercially available smoked salmon.[14,19,22] No differences in these parameters, nor in pH and ΔMt, were observed according to the employed salt formulation. Replacing NaCl partially by KCl implied a reduction in sodium content of approximately 37%.

The TVB-N and TMA-N mean values of the raw material were 126.0 and 48.4 mg N kg^{-1} of fish, respectively, which agrees with the values reported by other authors for fresh salmon.[19,26] The smoke-flavouring process slightly increased these parameters compared with the raw material. No differences were observed between the samples obtained with KCl-NaCl and the control samples.

The TBA index was used to evaluate the secondary products of lipid oxidation, which produce characteristic and undesirable off-odours.[27] In fish, these products come mainly from polyunsaturated fatty acid degradation. No malonaldehyde was detected in fresh salmon, so lipid oxidation was not remarkable. Low values were recorded for the recently smoke-flavoured samples (Table 2). No significant differences were found between salt types for this parameter.

The contents of inosine 5'-monophosphate, inosine, hypoxanthine and K_1-value of the fresh salmon and the recently smoke-flavoured fish are shown in Table 2. Neither the smoke-flavouring process nor salt formulation had a significant effect on any of these parameters.

The smoke-flavouring process reduced the growth of mesophilic and *Enterobacteriaceae* bacteria compared with fresh fish (Table 2). According to the low values found for volatile bases and microbial growth, the raw material and the recently smoke-flavoured salmon used in this study exhibited adequate hygienic quality.

No exudate was observed in the bags after the process. The liquid released by samples completely evaporated through the WP bags during the process as if there was no packaging. As demonstrated in previous studies, this method is a suitable alternative to traditional cold-smoking procedures since it enables good quality smoke-flavoured salmon to be obtained, while reducing product handling, brine waste and processing steps.[14,19]

Changes in physicochemical and microbiological quality during storage

The results of the pH, TBA index, TVB-N, TMA-N and shear force analyses in the smoke-flavoured salmon salted with KCl-NaCl and the control samples are shown in Fig. 2.

The pH values were not affected by salt formulation and remained nearly constant during storage, as observed in other studies into smoked fish.[28]

The TBA values progressively increased throughout storage for both sample types, which ranged from 0.08 to 0.46 mg MDA kg^{-1} fish. These values were below the limit reported by Connell[29] of 1-2 mg MDA kg^{-1} fish. Values above this limit can entail rancid flavour and odour.

TVB-N is a common indicator of spoilage for many fish species, which quantifies mainly ammonia, trimethylamine (TMA) and dimethylamine (DMA). The TVB-N value greatly depends on storage conditions, hygienic practices, processing types, etc.[26,30] The upper acceptability limits of spoilage for smoked fish fell within 300-400 mg N kg^{-1}.[31] In this study, the TVB-N concentration progressively increased throughout the storage period from 134 to 244 mg N kg^{-1} of fish (Fig. 2C), which agrees with other studies into cold-smoked salmon.[19] In contrast, the TMA-N values remained nearly constant throughout the study. In general, 100-150 mg kg^{-1} can be considered the upper limit for this parameter.[29,32] No sample reached the limits of acceptability proposed for TVB-N and TMA-N.

Fig. 2 shows the shear force test results of smoke-flavoured salmon during cold storage. Although the F max values oscillated during the study, no clear trend for this parameter was observed throughout storage, possibly due to the variability in thickness and/or lipid distribution among the fresh fish portions employed. Lipid content was typically distributed heterogeneously in the fish fillets, which affects texture to a great extent.[33,34] Minor differences were observed between salt types, but were not significant.

Changes in IMP, Ino and Hx of smoke-flavoured salmon for 42 cold storage days are shown in Fig. 3.

ATP degradation compounds provide information about the chemical changes that occur in fish flesh during storage, which have been used extensively as a freshness index.[35] A progressive drop in IMP content and an increase in Hx were observed for both sample types during storage. The maximum recorded Hx value was 3.47 µmol g^{-1}, which was below the limit of sensory rejection of 5-7 µmol g^{-1} set for smoked salmon according to Truelstrup-Hansen et al.[36] The Ino values were higher than Hx throughout the study, and showed that the degradation of Ino to Hx was limited. According to many authors,[31,32] degradation of IMP to Ino is attributed to autolytic enzymes, but accumulation of Hx in fish muscle is also connected to microbial spoilage. These results (no degradation of Ino to Hx) suggest limited microbiological activity, which is in agreement with the bacterial growth described below.

The K_1-value is a freshness ratio, obtained from concentrations of ATP breakdown products, that quantifies the extent of IMP degradation. The K_1-value increased moderately from 90.9 for both samples to 96% by the end of the study (data not shown). These high values were caused by the low IMP levels recorded from the beginning of the study as IMP degradation had occurred almost completely in the raw material. Similar K_1-value results have been reported for smoke-flavoured salmon by Rizo et al.[19] The partial replacement of NaCl with KCl did not affect the values of this parameter.

Spoilage flora in smoked fish is variable and complex, and often dominated by lactic acid bacteria and *Enterobacteriaceae*.[37,38] Mesophilic bacteria significantly increased in both samples during storage (Fig. 4A), without reaching the value established as the upper tolerable limit for cold-smoked fish (7 log cfu g^{-1}).[39] High levels of *Enterobacteriaceae* are related to poor hygienic practices during handling, and can determine the product's shelf-life.[40,41] Counts of *Enterobacteriaceae* were below the limits of quantification for these microorganisms (1 log cfu g^{-1}) almost throughout the study (Fig. 4B), which indicates a good hygiene level during smoke-flavouring. No differences in the evolution of these microorganisms were noted according to the salt used. These results agree with those of Fuentes et al.,[30] who found no differences for *Enterobacteriaceae* growth when smoked sea bass was salted with the 50% NaCl-50% KCl mixture or with 100% NaCl.

No differences were observed depending on sample type, which also occurred with the physicochemical parameters described above.

According to these results, the smoke-flavoured salmon obtained with the 50% KCl-50% NaCl mixture maintained good microbial and physicochemical quality throughout storage time. Therefore, it is suitable for human consumption for the studied 42-day period.

Multifactor analysis

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

The statistical analysis confirmed that storage time strongly influenced all the analysed parameters except for TMA-N, Ino and *Enterobacteriaceae*. In contrast, salt type did not affect any of the considered variables. In general, the interactions between factors were non-significant.

CONCLUSIONS

The maximum NaCl proportion that can be replaced with sodium-free (SF) salt was 50%. The smoke-flavoured salmon obtained by replacing 50% NaCl with either 50% SF salt or 50% KCl was perceived as being similar to the samples processed with 100% NaCl by the panellists. As using SF salt to replace NaCl offered no sensory advantage compared with using pure KCl, and as the price of SF salt is higher than KCl, it can be concluded that KCl was a better choice for replacing NaCl than SF salt.

During the storage study, no lipid oxidation was recorded in the smoke-flavoured samples, regardless of the salt type employed. TVB-N, TMA-N, mesophilic and *Enterobacteriaceae* increased similarly for both salt formulations, without exceeding the acceptance limits at any time of the study. The 50% replacement of NaCl with KCl did not cause major changes in the physicochemical parameters and shelf-life of smoke-flavoured salmon, which was over 42 days. The 50% NaCl replacement with KCl implied an approximate 37% reduction in sodium content in the smoke-flavoured salmon. This is an interesting option for reducing sodium content in such products to help meet the needs set by both health authorities and consumers.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the support of Tub-Ex Aps (Taars, Denmark) for suppling the water vapour permeable bags and for providing all the necessary technical information. Arantxa Rizo would like to thank the Universitat Politècnica de València for the FPI grant.

REFERENCES

1 World Health Organization, *Global status report on noncommunicable diseases 2014* http://apps.who.int/iris/bitstream/10665/148114/1/9789241564854_eng.pdf?ua=1 [1 May 2017].

2 European Commission, *National Salt Initiatives. Implementing the EU Framework for salt reduction initiatives, 2009* http://ec.europa.eu/health/nutrition_physical_activity/high_level_group/nutrition_salt_en.htm [5 May 2017].

3 Webster J, Trieu K, Dunford E and Hawkes C, Target Salt 2025: A Global Overview of National Programs to Encourage the Food Industry to Reduce Salt in Foods. *Nutrients* **6:** 3274–3287 (2014).

4 Toldrá F and Barat JM, Strategies for salt reduction in foods. *Recent Pat Food Nutr Agric* **4**: 19–25 (2012).

5 Bidlas E and Lambert RJW, Comparing the antimicrobial effectiveness of NaCl and KCl with a view to salt/sodium replacement. *Int J Food Microbiol* **124:** 98–102 (2008).

6 Boziaris IS, Skandamis PN, Anastasiadi M and Nychas GJE, Effect of NaCl and KCl on fate and growth/no growth interfaces of Listeria monocytogenes Scott A at different pH and nisin concentrations. *J Appl Microbiol* **102:** 796–805 (2007).

7 Pelroy GA, Scherer A, Peterson ME, Paranjpye R and Eklund MW, Inhibition of *Clostridium botulinum* type E toxin formation by potassium chloride and sodium chloride in hot processed (smoked) whitefish (*Oregonus slupeaformis*). *J Food Prot* **48:** 971–5 (1985).

8 Dos Santos BA, Campagnol PCB, Morgano MA and Pollonio MAR, Monosodium glutamate, disodium inosinate, disodium guanylate, lysine and taurine improve the sensory quality of fermented cooked sausages with 50% and 75% replacement of NaCl with KCl. *Meat Sci* **96:** 509–13 (2014).

9 Pietrasik Z and Gaudette NJ, The impact of salt replacers and flavor enhancer on the processing characteristics and consumer acceptance of restructured cooked hams. *Meat Sci* **96:** 1165–70 (2014).

10 Rodrigues MJ, Ho P, López-Caballero ME, Bandarra NM and Nunes ML, Chemical, microbiological, and sensory quality of cod products salted in different brines. *J Food Sci* **70:** M1–M6 (2005).

11 Tahergorabi R and Jaczynski J, Physicochemical changes in surimi with salt substitute. *Food Chem* **132:** 1281–1286 (2012).

12 Fuentes A, Fernández-Segovia I, Serra JA and Barat JM, Development of a smoked sea bass product with partial sodium replacement. *LWT - Food Sci Technol* **43:** 1426–1433 (2010).

13 Almli VL and Hersleth M, Salt replacement and injection salting in smoked salmon evaluated from descriptive and hedonic sensory perspectives. *Aquac Int* **21**: 1091–1108 (2012).

14 Rizo A, Máñes V, Fuentes A, Fernández-Segovia I. and Barat JM, A novel process for obtaining smoke-flavoured salmon using water vapour permeable bags. *J Food Eng* **149:** 44-50 (2015).

15 UNE-EN ISO 4120 *Sensory analysis. Methodology. Triangle test*, Asociación Española de Normalización y Certificación (AENOR), Madrid (2008).

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

17 Malle P and Tao SH, Rapid quantitative determination of trimethylamine using steam distillation. *J Food Prot* **50:** 756–760 (1987).

18 Tarladgis BG, Watts BM, Younathan MT and Dugan LR Jr, A distillation method for the quantitative determination of malonaldehyde in rancid foods. *J Am Oil Chem Soc* **37:** 44-48 (1960).

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

20 ISO 4833 *Microbiology of food and animal feeding stuffs. Horizontal method for the enumeration of microorganisms. Colony-count technique at 30ºC.* International Organization for Standardization, Geneva (2003).

21 ISO 21528-2 (2004) *Microbiology of food and animal feeding stuffs - Horizontal methods for the detection and enumeration of Enterobacteriaceae - Part 2: Colony-count method.* International Organization for Standardization, Geneva (2004).

22 Fuentes A, Fernández-Segovia I, Barat JM and Serra JA, Physicochemical characterization of some smoked and marinated fish products. *J Food Process Preserv* **34:** 83–103 (2010).

23 *Codex Alimentarius*, *Standard for smoked fish, smoke-flavoured fish and smoke-dried fish (311-2013).* FAO/WHO, Rome (2013).

24 Mitchell M, Brunton NP and Wilkinson MG, Current salt reduction strategies and their effect on sensory acceptability: a study with reduced salt ready-meals. *Eur Food Res Technol* **232:** 529–539 (2011).

25 Barat JM, Pérez-Esteve E., Aristoy MC and Toldrá F, Partial replacement of sodium in meat and fish products by using magnesium salts. A review. *Plant Soil* **368**: 179–188 (2013).

26 Fernández-Segovia I, Fuentes A, Aliño M, Masot R, Alcañiz M and Barat JM, Detection of frozen-thawed salmon (*Salmo salar*) by a rapid low-cost method. *J Food Eng* **113:** 210–216 (2012).

27 Sohn JH and Ohshima T, Lipid Oxidation, Odour, and Colour of Fish Flesh, in *Handbook of Seafood Quality, Safety and Health Applications,* pp. 96–108 (2010).

28 Goulas AE and Kontominas MG, Effect of salting and smoking-method on the keeping quality of chub mackerel (*Scomber japonicus*): biochemical and sensory attributes. *Food Chem* **93:** 511–520 (2005).

29 Connell JJ, *Control of fish quality* (4th ed.). Fishing News Books Limited, London (1995).

30 Fuentes A, Fernández-Segovia I, Barat JM and Serra JA, Influence of sodium replacement and packaging on quality and shelf-life of smoked sea bass (*Dicentrarchus labrax L.*). *LWT - Food Sci Technol* **44:** 917–923 (2011).

31 Dalgaard, P. (2000). Freshness, quality and safety in seafood. *Flair-Flow Europe Technical Manual F-FE 380A/00.* http://flairflow4.vscht.cz/seafood00.pdf. [2 May 2017].

32 Huss HH, Quality and quality changes in fresh fish. in *Fisheries technical paper, No. 348*, FAO, Rome (1995).

33 Ginés R, Valdimarsdottir T, Sveinsdottir K and Thorarensen H, Effects of rearing temperature and strain on sensory characteristics, texture, colour and fat of Artic charr (*Salvelinus alpinus*). *Food Qual Prefer* **15:** 177-185 (2004).

34 Katikou P, Hughes SI and Robb DHF, Lipid distribution within Atlantic salmon (*Salmo salar*) fillets. *Aquaculture* **202:** 89–99 (2001).

35 Fernández-Segovia I, Escriche I and Serra JA, Evolution of volatile fraction and ATP related compounds during storage of desalted cod (*Gadus morhua*). *Food Sci Technol Int* **14:** 37–47 (2008).

36 Truelstrup-Hansen L, Gill TA and Huss HH, Effects of salt and storage temperature on chemical, microbiological and sensory changes in cold-smoked salmon. *Food Res Int* **28:** 123–130 (1995).

37 Løvdal T, The microbiology of cold smoked salmon. *Food Control* **54:** 360–373 (2015).

38 Joffraud JJ, Cardinal M, Cornet J, Chasles JS, Léon S, Gigout F and Leroi F, Effect of bacterial interactions on the spoilage of cold-smoked salmon. *Int J Food Microbiol* **112:** 51–61 (2006).

39 ICMSF International Commission on Microbiological Specifications for Foods. Sampling plans for fish and shellfish, in *Microorganisms in foods. Sampling for microbiological analysis: Principles and scientific applications*, ICMSF (Ed.). Toronto, Canada: University of Toronto Press, pp. 181-196 (1986).

40 Gram L and Huss HH, Microbiological spoilage of fish and fish products. *Int J Food Microbiol* **33:** 121–37 (1996).

41 Leroi F, Joffraud JJ, Chevalier F and Cardinal M, Research of quality indices for cold-smoked salmon using a stepwise multiple regression of microbiological counts and physico-chemical parameters. *J Appl Microbiol* **90:** 578–87 (2001).

Table 1. Physicochemical parameters of the smoke-flavoured salmon samples obtained with 100% NaCl and by partial NaCl replacement (50% SF-50% NaCl and 50% KCl-50% NaCl). (Means and standard deviations, *n=3*).

		Smoke-flavoured salmon			
	Fresh salmon	**NaCl**	**SF-NaCl**	**KCl-NaCl**	**α**
Moisture (g H_2O kg^{-1})	$687±2^a$	$590±40^b$	$610±10^b$	$610±40^b$	**
z^{Cl^-} (g Cl^- mL^{-1})	-	$0.037±0.003^a$	$0.034±0.004^a$	$0.034±0.004^a$	ns
Na^+ (mg kg^{-1})	$590±70^a$	$15290±1000^c$	$9100±500^b$	$9640±870^b$	***
K^+ (mg kg^{-1})	$2990±130^a$	$2890±420^a$	$15350±1170^b$	$16250±2990^b$	***
a_w	$0.990±0.003^a$	$0.945±0.003^b$	$0.953±0.003^b$	$0.949±0.009^b$	***
ΔMt	-	$-0.110±0.009^a$	$-0.10±0.03^{bc}$	$-0.10±0.01^b$	*

z^{Cl^-}: Cl^- concentration in liquid phase; ΔMt: weight loss
Different letters in the same row indicate significant differences.
ns: no significant * $p<0.05$, ** $p<0.01$, *** $p<0.001$

Table 2. Physicochemical and microbiological parameters of fresh and recently smoke-flavoured salmon (day 0) salted with 100% NaCl and by 50% KCl-50% NaCl. (Means and standard deviations, *n=3*).

		Smoke-flavoured salmon		**α**
	Fresh salmon	**NaCl**	**KCl-NaCl**	
Moisture (g H_2O kg^{-1})	$720±10^a$	$610±20^b$	$610±20^b$	***
z^{Cl^-}(g Cl^- mL^{-1})	-	$0.037± 0.003^a$	$0.038±0.006^a$	ns
Na^+ (mg kg^{-1})	$450±250^a$	$16540±2650^c$	$10400±2530^b$	***
K^+ (mg kg^{-1})	$2940±120^a$	$2380±1010^a$	$14930±3180^b$	***
pH	$6.58±0.02^a$	$6.27±0.06^b$	$6.25±0.08^b$	***
a_w	$0.991±0.003^a$	$0.934±0.010^b$	$0.936±0.010^b$	***
ΔM_t	-	$-0.09±0.01^a$	$-0.08±0.05^a$	ns
Lipid (g kg^{-1})	$98.1±0.3^a$	$120±10^a$	$120±40^a$	ns
TBA (mg MDA kg^{-1})	nd	$0.08±0.06^a$	$0.07±0.03^a$	ns
TVB-N (mg N kg^{-1})	$126±5^a$	$134±9^{ab}$	$150±10^b$	*
TMA-N (mg N kg^{-1})	$48.4±0.6^a$	$60±4^b$	$56±1^b$	*
IMP (µmol g^{-1})	$0.74±0.09^a$	$0.9±0.2^a$	$1.1±0.6^a$	ns
Ino (µmol g^{-1})	$7±1^a$	$7±1^a$	$8±1^a$	ns
Hx (µmol g^{-1})	$1.3±0.1^a$	$1.2±0.1^a$	$1.4±0.1^a$	ns
K_1- value (%)	$91.5±0.2^a$	$90.9±0.9^a$	$90.9±0.9^a$	ns
Mesophilic (log cfu g^{-1})	$4.50±0.03^a$	$2.6±0.5^b$	$2.9±0.2^b$	***
Enterobacteriaceae (log cfu g^{-1})	2.1±0.2	nd	nd	

z^{Cl^-}: Cl^- concentration in the liquid phase; ΔMt: weight loss; TBA: thiobarbituric acid index; MDA: malonaldehyde; TVB-N: total volatile basic nitrogen; TMA-N: trimethylamine nitrogen; IMP: inosine-5'-monophosphate; Ino: inosine; Hx: hypoxanthine.

Different letters in the same row indicate significant differences. nd: not detected.

ns: not significant, * $p < 0.05$, *** $p < 0.001$

Table 3. F-ratio values and significance levels obtained in multifactor ANOVA for the microbiological and physicochemical parameters according to the factors: salt type (S), storage time (t) and their interaction (S x t).

	S	**t**	**S x t**
pH	0.44^{ns}	4.33^{*}	0.31^{ns}
TBA	0.90^{ns}	7.56^{***}	0.49^{ns}
TVB-N	2.96^{ns}	27.02^{***}	0.39^{ns}
TMA-N	4.26^{ns}	1.65^{ns}	0.44^{ns}
IMP	0.05^{ns}	5.66^{**}	0.31^{ns}
Ino	0.05^{ns}	1.30^{ns}	1.06^{ns}
Hx	3.81^{ns}	11.89^{***}	0.36^{ns}
K_1-value	0.35^{ns}	21.74^{***}	2.07^{ns}
Shear force	0.21^{ns}	3.42^{*}	1.50^{ns}
Mesophilic bacteria	0.52^{ns}	19.43^{***}	0.41^{ns}
Enterobacteriaceae	0.66^{ns}	2.21^{ns}	2.84^{*}

TBA: thiobarbituric acid index; TVB-N: total volatile basic nitrogen; TMA-N: trimethylamine nitrogen; IMP: inosine-5'-monophosphate; Ino: inosine; Hx: hypoxanthine.
ns: not significant * p <0.05, ** p <0.01, *** p <0.001

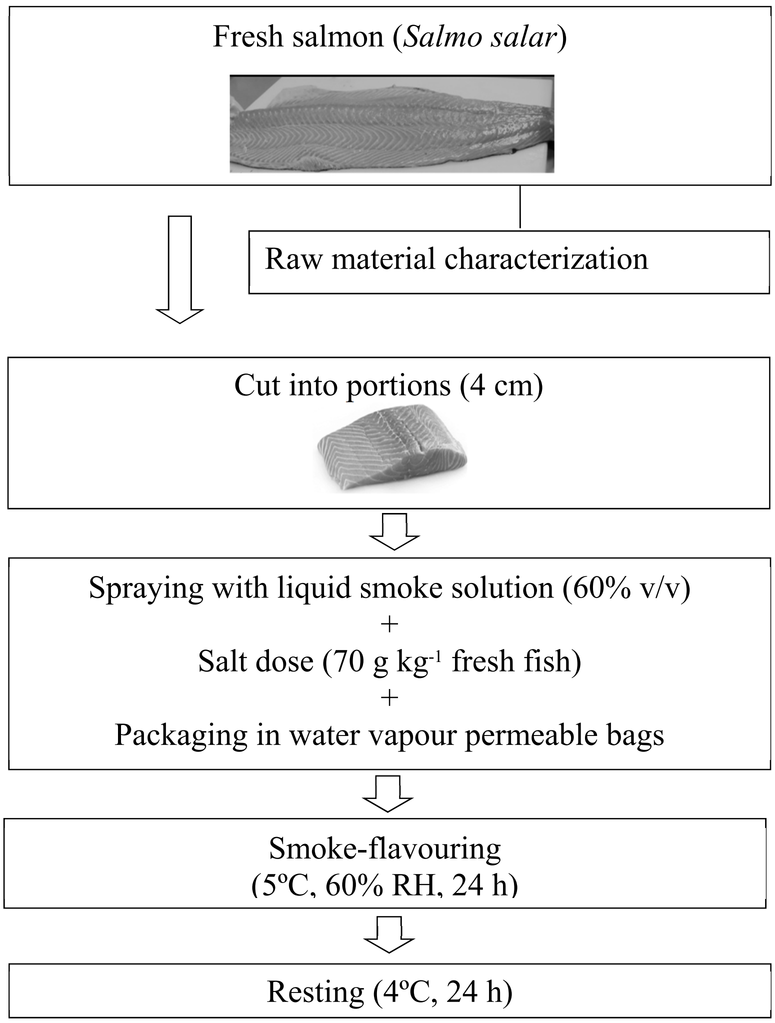

Figure 1. Smoke-flavouring process of salmon.

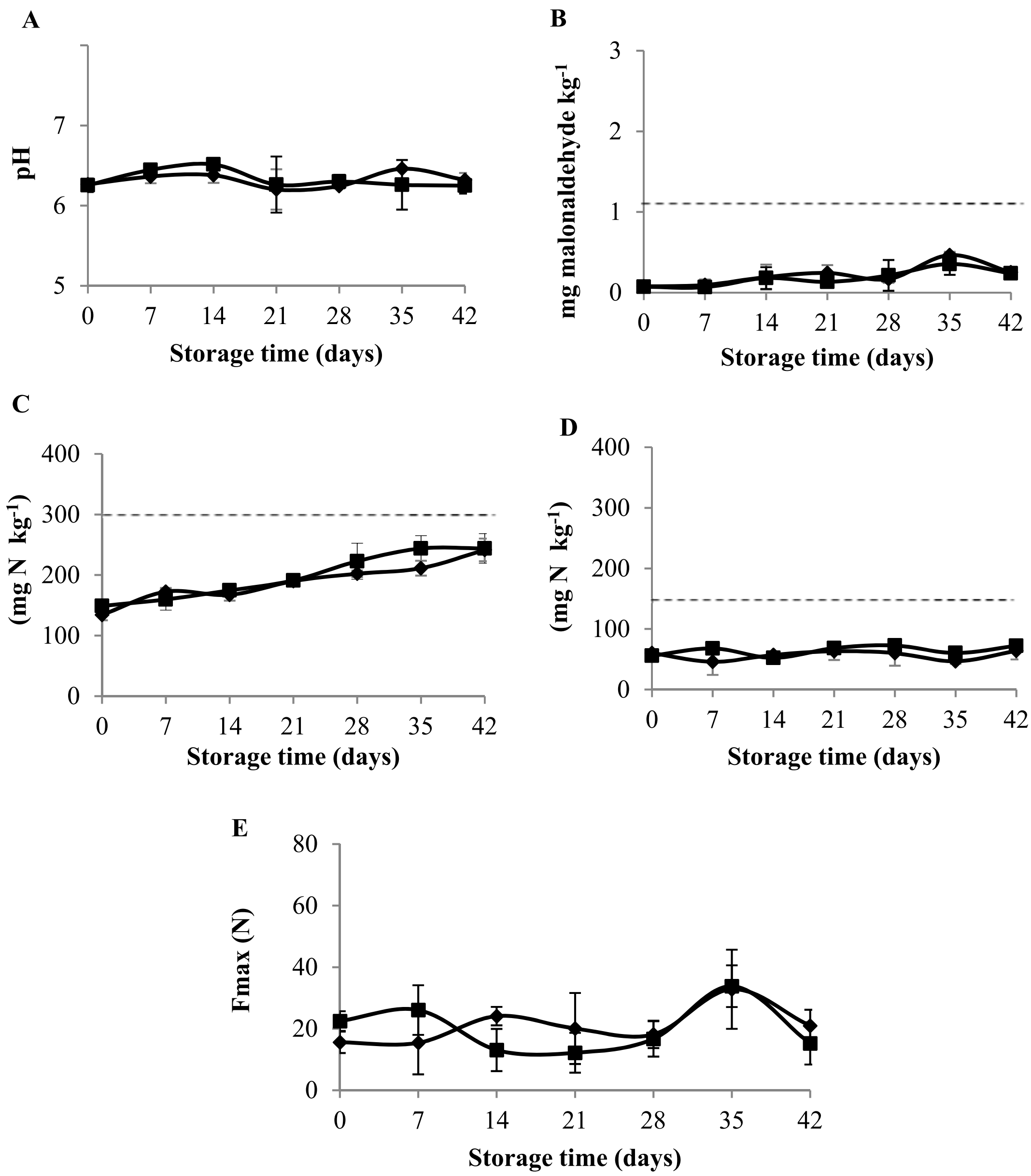

Figure 2. Evolution of pH (A), TBA index (B), TVB-N (C), TMA-N (D) and shear force (E) in samples of smoke-flavoured salmon obtained with different salt formulations (100% NaCl (◆) and 50% KCl-50% NaCl (■) during 42 days of storage at 4 ºC. (Means and standard deviations, *n=3*). Bars indicate the standard deviation. The dashed line represents unacceptable levels in each figure.

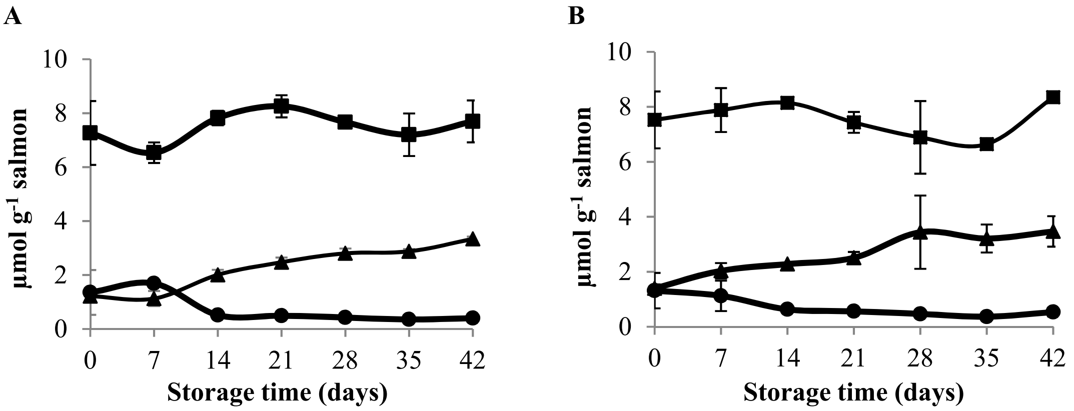

Figure 3. Evolution of inosine-5'-monophosphate (IMP) (●), inosine (Ino) (■) and hypoxanthine (Hx) (▲) in samples of smoke-flavoured salmon obtained with different salt formulations (100% NaCl (A) and 50% KCl-50% NaCl (B)) during 42 days of storage at 4 ºC. (Means and standard deviations, *n=3*). Bars indicate the standard deviation.

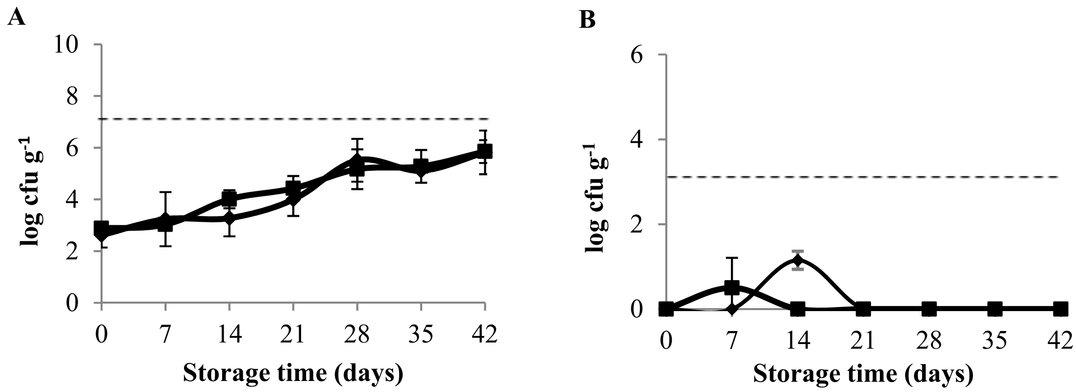

Figure 4. Evolution of mesophilic bacteria (A) and *Enterobacteriaceae* (B) in samples of smoke-flavoured salmon obtained with different salt formulations (100% NaCl (◆) and 50% KCl-50% NaCl (■)) during 42 days of storage at 4 ºC. (Means and standard deviations, *n=3*). Bars indicate the standard deviation. The dashed line represents unacceptable levels in each figure.