Development of a novel smoke-flavoured trout product: an approach to sodium reduction and shelf life assessment

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Abstract

This work aimed to develop a reduced sodium smoke-flavoured trout product with similar physico-chemical traits and sensory quality to commercial smoked trout. In a first phase, a reduced sodium smoke-flavoured trout product was developed by a novel smoke-flavouring process using water vapour permeable bags. In a second phase, the obtained product's microbial and physico-chemical quality was evaluated for 42 cold storage days. A smoke-flavoured trout product with similar physico-chemical characteristics and sensory acceptance to commercial smoked trout was achieved through smoke-flavouring with water vapour permeable bags. Partial substitution of NaCl for KCl led to a 42% sodium reduction in the smoke-flavoured trout and did not affect its physico-chemical traits, sensory attributes and hygienic quality throughout the storage. During shelf life study, no sample exceeded the limits of acceptance proposed for physico-chemical and microbial parameters, except for mesophilic bacteria, which limited the product shelf life to 1 month.

Keywords: Smoke flavouring; trout; NaCl; KCl; water vapour permeable bags; shelf life.

1. Introduction

Fish smoking techniques involve a salting step prior to smoking, which is essential in preservation, texture and product flavour terms. However, processed foods like “ready-to-eat” fish products are considered important contributors to dietary salt intake, which is linked to increased risk of cardiovascular disease. Some countries have implemented control measures, such as mandatory labelling for such products as “highly salted” to promote consumer awareness (WHO, 2009). Health authorities’ efforts to encourage low-sodium diets and increase fish intake render the development of less salty fish products is a relevant issue.

To achieve this goal, the food industry is attempting to reformulate recipes to reduce the sodium of its products, while maintaining food safety and consumer acceptance. The main strategy to adopt in order to reduce the sodium in these foodstuffs consists in the partial replacement of NaCl with other salts (KCl, $CaCl_2$, $MgCl_2$, K-lactate, etc.). Partially substituting NaCl for KCl is the best alternative to reduce sodium content, but the main limitation of using KCl is the bitter and metallic flavour that it confers foods if used at high levels (Toldrá and Barat, 2012), and 50:50 NaCl/KCl blends are the common practical industrial limit. However, this limit can vary depending on the type of food and presence of other ingredients, such as spices or smoke flavours, which can mask the residual flavours associated with using KCl. Hence the sodium substitution level in smoked products could be higher than in other kinds of food matrices (Fuentes et al., 2012; Mitchell et al., 2011).

In this context, a new methodology to obtain smoke-flavoured salmon based on a controlled salted process and the use of water vapour permeable (WP) bags has been developed (Rizo et al., 2015a). The process was found to effectively reduce handling, brine waste and processing steps without affecting the smoke-flavoured fish's sensory acceptance and the physico-chemical quality (Rizo et al., 2016a, 2016b).

Rainbow trout (*Oncorhynchus mykiss*) is one of the most produced aquacultured fish in Europe (FAO, 2014). Lower stable market prices, and its smaller whole "easy-to-handle" fillets, make trout a more profitable raw material for smoking than Atlantic salmon, especially given the close resemblance between the commercial smoked products of both species (Salánet al., 2006).

Thus we considered that a combined approach that would integrate partial NaCl replacement into the described smoke-flavouring process would provide high added value to smoke-flavoured trout products, which could meet the needs of both consumers and producers, who demand healthier fish products and improved process yields.

The objectives of this study were to: (a) develop a reduced sodium smoke-flavoured trout product by the new smoke-flavouring process; (b) evaluate the obtained product's physico-chemical and microbial quality during storage.

2. Material and methods

2.1. Materials

Aquacultured trout (*Oncorhynchus mykiss*) were obtained from Piscifactorias Andaluzas, S.A (Granada, Spain). Fish specimens (commercial weight 300-700 g) were transported to the laboratory in polyspan boxes with ice and then stored at 4 ºC until processing. were purchased from a local market in the city of Valencia (Spain) Trout were headed and gutted, and fillets were trimmed to remove bones before processing. Eighty-six trout fillets (average weight 111±25 g) were employed for the complete test (38 for the first phase and 48 for the second).

NaCl and KCl salts were supplied by Panreac Química, S.A. (Barcelona, Spain) and natural liquid smoke HARDWOOD AFS 10 was provided by Amcan Ingrédients Ltd., Le Chesnay, France). The water vapour permeable bags (WP) used for smoking-salting were supplied by TUB-EX ApS (Taars, Denmark) (polyamide mix; size: 200×300×0.04 mm; water vapour transmission rate: 5.0 g/50 μ/m²/24 h (38 °C/50% RH).

Two smoked trout batches of three different brands were analysed to establish the target smoke-flavoured trout's physico-chemical parameters (moisture, salt content and a_w). Commercial samples were purchased in local supermarkets, transported to the laboratory and analysed 15 days before expiration date. The commercial samples were, at distribution point, sliced, vacuum packed, and stored at 4 °C. Pack weights was 100 g and the ingredients given in the labels were the same in all the cases: trout, salt and natural smoked. Raw material of these products was aquacultured rainbow trout from different EU countries (Norway, Denmark, and France). Fish was processed using traditional cold-smoking techniques. The raw material of these products was aquacultured rainbow trout, processed according to traditional cold-smoking techniques: dry salting, followed by a smoking step in a smoking chamber.

All the reagents and culture media were provided by Scharlau Chemie, S.A. (Barcelona, Spain).

2.2. Experimental design

2.2.1. Phase I: Developing a reduced sodium smoke-flavoured trout product

The optimal conditions for obtaining smoke-flavoured trout were established by studying the effect of the amount of salt doses and processing time on the final product physico-chemical properties. These conditions were set to obtain smoke-flavoured trout with similar characteristics to currently marketed products. The values considered as reference were obtained from the analysed commercial products.

Trout fillets were subjected to a simultaneous smoking-salting procedure based on the use of water vapour permeable (WP) bags (Fig. 1a) following the method developed by Rizo et al. (2015a). Diluted liquid smoke was applied to fish by spraying fish surface for 30 s. In this phase, three salt dose concentrations were considered, 2, 4, and 6 g /100 g salt fresh trout, as were two processing times, 12 h and 24 h. Then trout samples were vacuum-packaged (Tecnotrip mod. EV-25-CD, Barcelona, Spain) in highly water vapour permeable (WP) bags. It should be noted that vacuum packaging was used merely to ensure good initial contact between fish and the WP bag. The smoke-flavouring process was carried out at 60% relative humidity (RH) and 5 ºC in a drying chamber (Binder mod. KBF. Tuttlingen, Germany). After the processing time, trout samples were removed from the bags and were placed in saturated brine under constant stirring for 30 s to remove any traces of salt attached to surfaces. Finally, fillets were dried with absorbent paper and weighed. The obtained smoke-flavoured trout was characterised by analyses of moisture, chloride content, a_w and weight loss (ΔM_t). The sensory acceptance of the obtained products was also evaluated.

After establishing the appropriate processing conditions (4 g/100 g of salt dose, 24 h), the sodium reduction approach was applied (Fig. 1b). Trout fillets were processed by using a salt mixture of 50% KCl-50% NaCl (w/w) and 100% NaCl (control samples). The percentage of substitution was selected according to the results obtained in a previous work (Fuentes et al., 2011), which concluded that NaCl can be replaced with up to 50% KCl without affecting the smoke-flavoured fish sensory and physico-chemical traits. The obtained samples were characterised by physico-chemical and microbiological analyses, and a sensory test was conducted.

2.2.2. Phase II: Physico-chemical and microbial quality during storage

The objective of the second phase was to evaluate the quality and shelf life of the novel reduced sodium smoke-flavoured trout fillets obtained in Phase I. For this purpose, samples were vacuum-packaged and stored for 42 days at 4 ºC. The physico-chemical and microbiological analyses were performed on the smoke-flavoured products (reduced-sodium and control) on cold storage days 0, 7, 14, 21, 28, 35 and 42. On each sampling day, three bags were analysed by salt formulation (n=3). Duplicate analyses were performed on each sample, except for pH, which was measured in quintuplicate.

2.3. Analytical determinations

2.3.1. Physico-chemical analyses

Moisture and lipid content were determined in accordance with AOAC methods 950.46 and 991.36, respectively (AOAC, 1997). Chloride content was determined 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 determine sodium and potassium contents by absorption spectrophotometry using a Perkin-Elmer spectrophotometer, model 3100 (Norwalk, CT, USA). pH measurements were taken by a micropH 2001 digital pH-meter (Crison Instruments, S.A., Barcelona, Spain) with a puncture electrode (Crison 5231) at five different locations on the fish fillets. Water activity (a_w) was measured with an 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 according to the method described by Malle and Tao (1987). The thiobarbituric acid (TBA) index was measured by a spectrophotometric method with some minor modifications (Tarladgis et al., 1960), results are expressed as mg malonaldehyde (MDA) / kg fish sample.

2.3.2. Texture measurements

A texture profile analysis (TPA) and a shear force test were performed on the smoke-flavoured trout fillets with a Texture Analyser TA.XT2® (Stable Micro Systems, Surrey, UK) equipped with a load cell of 250 N. Previously skinned fillets were cut to obtain parallelepiped pieces (3 x 2 cm) from the dorsal part of fillets. Measurements were taken of the samples at room temperature.

For the TPA analysis, a flat-ended cylindrical plunger SMS P75 (75 mm diameter)was employed. This plunger was pressed into the sample at a constant speed of 1 mm/s until it reached 50% of sample height. Force-distance curves were processed to obtain hardness, chewiness, adhesiveness, springiness, cohesiveness and resilience parameters.

For the shear force test a HDP/BS Warner-Bratzler cell was used, which sliced the samples perpendicularly to the muscle orientation at a constant speed of 1 mm/s using a 90º angle inverted knife. Shear force was determined by the maximum force (N) recorded.

2.3.3. Colour determinations

Colour determination was performed in the flesh of trout fillets. A Minolta CM-700-d photocolorimeter (Minolta, Osaka, Japan) was used, equipped with a 10° observer and illuminant D65. Using the CIE L*a*b* coordinates the overall colour differences (ΔE) of the recently smoke-flavoured samples (day 0) compared with each storage study sampling day were determined.

2.4. Microbiological analyses

Mesophilic bacteria and *Enterobacteriaceae* were determined according to the methods standards provided by ISO (ISO, 2003, 2004a), respectively.

2.5 Sensory analyses

A sensory assessment was made to determine the smoke-flavoured trout product sensory acceptance. This test was conducted with the smoke-flavoured trout samples obtained under different processing conditions (salt dose: 2, 4, 6 g/100 g NaCl; processing time: 12 h and 24 h) and with a commercial smoked trout sample. Attributes like appearance, colour, odour, smoke odour, taste, saltiness and global acceptance were evaluated. Tests were done on semi-structured scales with 8 cm lines and three anchor points (0 = unpleasant, 4 = acceptable, and 8 = pleasant) for all the attributes, except for smoke odour and saltiness, where the anchors corresponded to insufficient, optimum and excessive (0, 4, and 8, respectively). A selected trained panel of seven assessors with experience in smoked fish assessment performed the sensory evaluation. Two sessions (one per processing time) were conducted, during which panellists were served four randomised samples on the same dish (3 smoke-flavoured trout samples and 1 commercial sample).

A triangle test (ISO, 2004b) was carried out to test for similarity between the reduced smoke-flavoured trout and the control samples (100% NaCl). Test sensitivity, given by α and β-risk was established as 0.05. The sensory assessment was made by 66 untrained panellists, who received a set of three samples and were informed that two were alike and one was different. They were asked to report which sample they believed to be different.

2.6. Statistical analysis

Statistical treatment of the data was performed using the Statgraphics Centurion software (Statpoint Technologies, Inc., Warrenton, VA, USA). In Phase I, a one-way ANOVA was conducted to discriminate among means. The least significant difference (LSD) procedure was used to test for the differences between averages at the 5% significance level. During the storage study, physico-chemical and microbiological data were analysed with a multifactor ANOVA to evaluate the effect of salt formulation and storage time. Tukey´s test procedure was used to test for any differences between means ($p<0.05$).

3. Results and discussion

3.1 Phase I: developing a reduced sodium smoke-flavoured trout product

3.1.1. Smoking-salting process optimisation

The physico-chemical parameters of the commercial smoked trout of three different brands were used to establish the reference values for the smoke-flavoured trout product (Table 1).

Wide variability was found among the physico-chemical parameters of all three brands, which occurred with other studies reported for smoked fish (Cornu et al., 2006). Given the differences found among brands, the average of the three brands was established as a reference value (62 g /100 g H_2O, 3.4 g 100 g NaCl/, a_w =0.957).

To achieve a smoke-flavoured trout product with similar physico-chemical traits to those established as a reference of commercial products, three salt doses (2, 4 and 6 g/100 g salt) and two processing times (12 h and 24 h) were tested.

As expected, the smoke-flavouring process significantly lowered the moisture and a_w values, and salt concentration increased compared with fresh trout (Fig. 2). The longer the processing time and the higher the salt dose, the lower the moisture and water activity. Salt content in the smoke-flavoured samples increased with salt dose, but a longer processing time did not significantly affect salt concentration. In contrast, weight loss was affected by processing time, but not by salt dose, which agrees with previous results from a similar study done with smoke-flavoured cod (Rizo et al., 2016a). This could be due to the fact that a higher salt dose led to a higher water loss and a higher salt gain, therefore salt dose does not affect total weight loss.

After the process, no exudate was collected from the bags of any samples as the WP bags were permeable enough to allow all the water released by fish muscle to completely evaporate, which thus reduced brine waste. Similar results have been reported for salmon and cod obtained by smoke flavouring with WP (Rizo et al., 2015a; 2016a). These results confirmed that trout can be salted, dried and smoked in a single step inside WP bags by a controlled process. This implies reducing not only brine waste, but also the handling and processing steps, compared with traditional methods in which salting, drying and/or smoking are carried out separately.

Of all the tested conditions, salting with 4 g/100 g NaCl for 24 h produced a smoked trout product that had the closest physico-chemical values to the reference ones.

The NaCl content of these samples corresponded to a sodium chloride content of 5.7 g/100 mL NaCl the liquid phase. This value fulfilled the Codex standard for smoked fish, smoke-flavoured fish and smoked dried fish (Codex, 2013), in which a minimum content of 5 g/100 mL NaCl is required to ensure complete protection against *Clostridium botulinum* at storage temperatures between 3°C and 10°C. This salt content would ensure an a_w value equal or lower than 0.97 in the food product. FSA guideline suggests that if

a_w is the controlling factor for safety, an a_w of 0.97 or below should be achieved throughout all components of the food product (ESA, 2017). Different studies have established that that apart from the reduced aw, there is no added bacteriostatic or bactericidal effect, imposed by the different cations of Na+ or K+. Therefore, NaCl can be replaced by KCl without risking the microbiological safety (Bildas and Lambert, 2008).

An acceptance test was carried out to check the acceptability of the obtained products. The smoke-flavoured trout samples obtained by the novel methodology and commercial smoked trout (brand 3) as a control sample were evaluated (Table 2).

All the samples obtained scores above 4 for all the evaluated attributes, which indicates that sensory acceptance was satisfactory. The smoke-flavoured samples generally obtained a higher score for appearance, colour and odour than the commercial samples, regardless of processing time and salt dose. Regarding saltiness, the samples processed with a 6 g/100 g salt dose and the commercial samples scored furthest from the optimal value for all the processing times. The panellists considered that the saltiness of these samples was excessive. For taste and global acceptance, the samples processed for 24 h scored higher than the commercial samples, but the samples processed for 12 h obtained lower acceptability scores. This evaluation revealed that the smoke-flavoured trout sensory attributes were perceived with the same degree of acceptance as the commercial smoked trout. This finding indicates that this methodology is suitable for obtaining a smoke-flavoured trout product with adequate sensory quality. These results are consistent with those reported for the smoke-flavoured salmon obtained by the same technique (Rizo et al., 2015a).

According to the sensory and physico-chemical results, a processing time of 24 h and a salt dose of 4 g/100 g were selected to develop reduced sodium smoke-flavoured trout.

3.1.2. Developing reduced sodium products

Table 3 shows the recently analysed parameters in the smoke-flavoured trout in which NaCl was replaced with KCl and the control samples were salted with 100% NaCl.

The moisture, chloride content and a_w of the obtained smoke-flavoured products were similar to the reference values established in Phase I, and fulfilled the minimum salt content (z^{NaCl}=5 g/100 mL salt) expressed as chloride content (z^{Cl-}= 3 g/100 mL salt), as set out by the above-mentioned standard for smoked fish, smoke-flavoured fish and smoked dried fish (*Codex*, 2013). No significant differences in these parameters, or in ΔMt and pH, were observed according to the salt formulation used. The obtained results showed that the mixture of salts employed allowed an approximate 42% reduction of sodium content compared with the control samples.

The TBA index was used to evaluate the secondary lipid oxidation products which produce characteristic and undesirable off-odours. Similar values were recorded for both sample types (0.12 mg MDA/kg), which were lower than those reported in other studies into smoked fish (Bugueño et al., 2003; Fuentes et al., 2011). These lower values could be related to the refrigeration temperatures employed throughout the process (5ºC) as processing temperatures influence the formation of secondary oxidation compounds (Espe et al., 2002; Goulas and Kontominas, 2005)

As with the TBA index, no differences were observed in the TVB-N and TMA-N values, mesophilic bacteria and *Enterobacteriaceae* between the samples obtained with KCl-NaCl and the control samples. According to these results, the recently trout smoke-flavoured product offered adequate hygienic quality. Salt replacement did not affect trout texture, except for chewiness which obtained higher values for the KCl-NaCl samples (Table 3). Regarding colour, partial NaCl substitution had no significant effect on the recently smoke-flavoured samples. Liquid smoke application on fish, and also the initial variability among the fresh fish fillets employed, reduce the possible differences that using different salts could have (Fuentes et al., 2012). No exudate was observed in any of the bags because, as described above, the liquid released by samples evaporated completely through the WP bags during the process.

A triangle test for similarity was carried out to check if there were any perceptible differences between the samples salted with KCl-NaCl and the control samples. According to the results obtained (23 correct responses of 66 evaluations), no more than 20% of the consumers were able to detect differences between the samples with a confidence level of 0.05 (α and β-risk). The use of high replacement levels of NaCl with KCl above 40-50% can diminish flavour intensity and produce bitter tastes, but the replacement level varies according to food product type, and presence of significant levels of smoke flavours and spices can help mask the bitter taste conferred by K^+ (Mitchell et al., 2011).

3.2. Phase II: physico-chemical and microbial quality during storage

Figure 3 illustrates the evolution of the TBA index, TVB-N, TMA-N, mesophilic bacteria and *Enterobacteriaceae* in the smoke-flavoured samples.

The TBA values increased for both sample types throughout storage. Samples obtained with KCl-NaCl displayed lower lipid oxidation than control samples. The values of both sample types remained generally lower than the limits proposed by Connell (1995) of 1-2 mg MDA/kg of fish flesh, at which fish can develop an objectionable odour. So shelf life was not limited by lipid oxidation.

TVB-N is a common indicator of spoilage for many fish species. No sample exceeded the upper limit of acceptability of spoilage established for smoked fish of 30-40 mg N/100 g

(Dalgaard, 2000). In this study, the TVB-N concentration increased throughout the storage period from 14 to 23 mg N/100 g of fish (Fig. 3), which agrees with the results reported by Alçiçek (2011) for liquid-smoked trout stored under vacuum conditions. The same tendency was found for TMA-N, for which 10-15 mg/100 g was the upper limit for this parameter (Connell, 1995). No differences were found between salt formulations in the TVB-N and TMA-N values, which remained far below the limits of acceptability previously mentioned (40 mg TVB-N/100 g and 15 TMA-N/100 g, respectively), throughout the study period.

Mesophilic bacteria significantly increased for all the samples during storage, but did not reach the value established as the upper tolerable limit for cold-smoked fish (7 log cfu/g) (ICMSF, 1986) until day 35. Some studies have found high mesophilic bacteria levels in cold-smoked salmon (10^7-10^8 cfu/g) before signs of spoilage became apparent, which sometimes make them unreliable as quality indicators of cold-smoked fish (Joffraud et al., 2006; Løvdal et al., 2015).

High levels of *Enterobacteriaceae* are related to poor hygiene practices during handling and can determine the shelf-life of the product (González-Rodríguez et al., 2002). *Enterobacteriaceae* counts remained below the limit of acceptability for these microorganisms (3 log cfu/g) throughout the study (FSA, 2017)., which indicates a good level of hygiene during smoking-salting. No differences in the evolution of these microorganisms during storage were recorded according to the salt used. This could suggest that partial sodium replacement did not affect microbial fish spoilage, which agrees with Fuentes et al. (2011), who found no differences for mesophilic bacteria and *Enterobacteriaceae* growth when liquid-smoked sea bass was salted with a 50% NaCl:50% KCl mixture and by 100% NaCl. Several studies have confirmed similar antimicrobial activity of KCl and NaCl at an equivalent a_w (Bidlas and Lambert, 2008;

Boziaris et al., 2007). According to these results, the shelf life of smoke-flavoured trout would be around 1 month, regardless of salt formulation.

Partial NaCl replacement and storage time did not result in any significant changes in the colour of samples, except for lightness (Table 4). The increase in L* coordinate could be attributed to water loss from samples during storage, which is retained in the plastic that covered the samples, as reported in other studies into smoked fish (Fuentes et al., 2012; Rizo et al., 2015b).

Texture profile analysis (TPA) and shear force test were carried out to determinate the effect of sodium replacement and storage time on the texture of fish muscle. In general, TPA parameters were not affected by the type of salt employed (Table 4). Only adhesiveness and springiness exhibited slight differences during the whole study; however there is not a clear tendency during the whole study. Similar results were obtained by Fuentes et al. (2012) who established that a partial sodium replacement did not affect the texture of smoked see bass.

Regards to shear force test, significant differences were observed depending on storage time and salt formulation; however, tendencies of changes were not uniform in any case. These values were in the range of those observed in different studies by other fish species with similar calibre and composition (Gómez-Estaca, et al., 2010).

4. Conclusions

Smoke-flavoured trout was achieved with similar physico-chemical characteristics and sensory acceptance to commercial smoked trout by means of the novel smoke-flavouring process using WP bags. Partial substitution of NaCl with KCl led to an approximate 42% sodium reduction of smoke-flavoured trout, and affected neither its physico-chemical characteristics, sensory features nor self-life under cold storage.

The smoke-flavouring process with WP bags is a fast convenient option to obtain high quality products, minimizing handling, and reducing processing steps and brine wastes. Moreover, this new method could be of interest to producers for reducing sodium content in fish products, which contributes to reductions in dietary sodium intake and satisfies the increasing consumer demands for these products.

Acknowledgements

The authors gratefully acknowledge the support of Tub-Ex Aps (Taars, Denmark) for the supply of 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.

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Captions

Fig 1. Trout smoking-salting (phase I): process optimisation (a). Developing the reduced sodium product (b)

Fig. 2. Moisture (a), NaCl content (b), water activity (c) and weight loss (ΔM_t) of the smoke-flavoured trout samples obtained by using different salt doses (2%, 4%, and 6% NaCl) and different processing times (12 h (grey colour) and 24 h (black colour)). Mean values±SD (n=3). Bars indicate standard deviation. The dashed line represents the reference value. Different lower case and capital letters indicate significant differences for the salt dose and processing times factors, respectively ($p < 0.05$).

Fig. 3. Evolution of the TBA index (a), TVB-N (b), and TMA-N (c), mesophilic bacteria (d), *Enterobacteriaceae* (e) of the smoke-flavoured trout samples obtained with different salt formulations (KCl-NaCl (black colour) and NaCl (grey colour)) for 42 storage days at 4ºC. Bars indicate standard deviation. The dashed line represents unacceptable levels in each figure. Different lower case and capital letters indicate significant differences for the salt type (S) and storage times (T) factors, respectively ($p < 0.05$)