LWT - Food Science and Technology 44 (2011) 917–923

Contents lists available at ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

Influence of sodium replacement and packaging on quality and shelf life of smoked sea bass (*Dicentrarchus labrax* L.)

Ana Fuentes, Isabel Fernández-Segovia*, José M. Barat, Juan A. Serra

Departamento de Tecnología de Alimentos, Universidad Politécnica de Valencia, Camino de Vera s/n, 46022 Valencia, Spain

ARTICLE INFO

Article history:
Received 10 May 2010
Received in revised form
16 November 2010
Accepted 23 November 2010

Keywords:
Smoked sea bass
Partial sodium replacement
Modified atmosphere
Vacuum packaging
Shelf life

ABSTRACT

The aim of this work was to study the effect of sodium chloride replacement by potassium chloride on the quality of smoked sea bass, as well as the effect of different types of packaging. Samples were salted with 100% NaCl or 50% NaCl-50% KCl, then smoked, and packaged in air, vacuum, or modified atmosphere. Chemical, microbial, and sensory analyses were periodically carried out during cold storage. In general, partial sodium replacement did not affect total volatile basic nitrogen, trimethylamine nitrogen, thiobarbituric acid, microbial counts, or sensory scores. However, the formation of histamine, putrescine, and cadaverine was delayed by using the mixture of salts. Vacuum and modified atmosphere packaging increased samples shelf life compared with air.

1. Introduction

Salt is commonly employed in fish processing, because it helps increase shelf life and has an important effect on water holding capacity (WHC), fat binding, colour, flavour, and texture. However, dietary sodium intake is higher than recommended in developed countries. The current levels of sodium consumption as sodium chloride have been directly associated with a greater likelihood of increased blood pressure, which in turn has been directly related to the development of cardiovascular and renal diseases (He & MacGregor, 2002). For these reasons, national and international bodies have set targets for a reduction in sodium consumption (WHO, 2004). The partial substitution of NaCl by KCl seems to be the best alternative for reducing sodium content. Indeed both salts have similar properties and potassium intake has not been linked to the development of hypertension and cardiovascular diseases (Geleijnse et al., 2007). However, the use of KCl is mainly limited by its bitter and astringent taste (Reddy & Marth, 1991). Replacement of NaCl by more than 50% of KCl can detract from flavour intensity and produce bitter tastes (Hand, Terrell, & Smith, 1982). However, the replacement level varies according to the type of food product. To this end, several researchers have attempted in recent decades to develop acceptable low salt products using NaCl/KCl mixtures.

Smoking delays microbiological and oxidative changes and is a traditional method of preserving fish. The ability of the smoking process to preserve fish is due to the synergistic action of salt incorporation, the preservative effect of smoke compounds, and dehydration. However, the objective is not only to retard the action of bacteria and enzymes but also to tenderize or change the taste, texture, and structure of the raw material, creating a product with a characteristic flavour and an extended but limited shelf life. To preserve the intrinsic features of the product for a long time, cold storage and an efficient system of packaging are needed. In this sense, vacuum and modified atmosphere packaging (MAP), in combination with refrigeration, have been shown to extend shelf life of fish and fish products by retarding microbial growth (Sivertsvik, Jeksrud, & Rosnes, 2002).

The aim of this work was to study the effect of sodium chloride replacement by potassium chloride and different types of packaging on the microbial, chemical, and sensory quality of smoked sea bass in cold storage.

2. Materials and methods

2.1. Sample preparation

Aquacultured European sea bass (*Dicentrarchus labrax* L.) were purchased from a local market in Valencia (Spain). All fish specimens were headed, gutted, and filleted, and two fillets were

* Corresponding author. Tel.: +34 96 387 70 07x73664; fax: +34 963877369.
E-mail address: isferse1@tal.upv.es (I. Fernández-Segovia).

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doi:10.1016/j.lwt.2010.11.030

obtained from each fish. These fillets were then washed in running water and submitted to a previously defined smoking process consisting of three stages: salting, liquid smoke application, and drying (Fig. 1).

The fillets were randomly divided into two groups before the salting stage, one group was salted with NaCl (termed Na); and the other group with a mixture of NaCl/KCl/$MgCO_3$ (0.9:1:0.1 w/w/w) (termed Na:K). $MgCO_3$ was used as anticaking in order to keep the mixtures flowing freely. The salting and smoking conditions, as well as the proportion of Na replaced by K were established in a previous study (Fuentes, Fernández-Segovia, Serra, & Barat, 2010). All salts were supplied by Panreac Química SA (Barcelona, Spain).

Each group of samples (Na and Na:K) was randomly divided into three new batches, which were packaged under three different conditions: with air (A), in vacuum (VP), and in modified atmosphere (MAP) (CO_2/N_2, 70:30). All samples were stored at 4 °C during 42 days.

Chemical and microbiological analyses were performed at 7-day intervals during storage. Sensory analyses were carried out at days 0, 14, and 28 of storage.

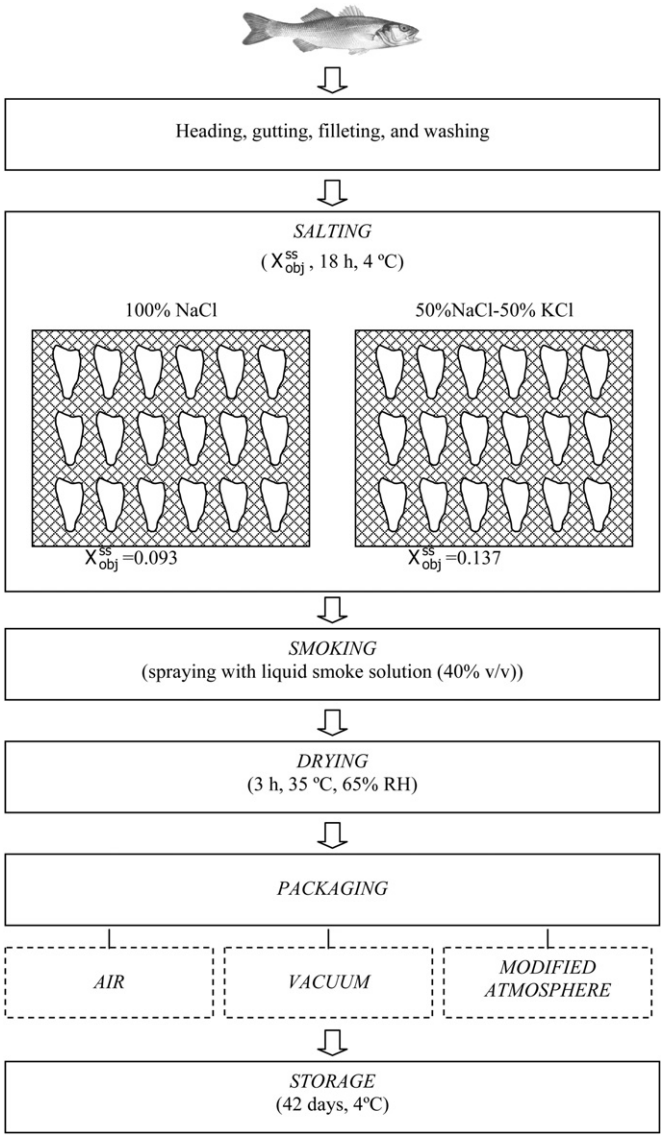

Fig. 1. Experimental design of the treatments carried out with the raw material.

2.2. Chemical analyses

Contents of TVB-N and TMA-N were determined by steam distillation according to the method described by Malle and Tao (1987). The TBA index was determined using a spectrophotometric method (Vyncke, 1970) to evaluate oxidation stability during chilled storage.

The determination of biogenic amines (BAs) was undertaken by HPLC with pre-column derivatization. Extraction of the BAs was conducted according to the method described by Venciana-Nogués, Vidal-Carou, and Mariné-Font (1995). For derivatization, 6-aminoquinolyl-N-hydroxysuccimidyl carbamate (AQC) contained in the kit AccQ-Fluor™ (Waters, Mildford, MA, USA) was employed following the instructions provided by the supplier. The derivatized biogenic amines were analyzed by reverse-phase HPLC in a Waters liquid chromatograph (model 2695) with a fluorescence detector (model 2475 from Waters, Mildford, MA, USA). Separations were achieved on a Waters XBridge™ C18 (4.5 × 150 mm, 5 μm particle size), thermostated at 55 ± 1 °C. The solvent system consisted of mobile phase A and B. Methanol LC-grade (JT Baker, Deventer, Holland) was used for mobile phase A; and 20 mmol/L of sodium acetate in ultra-high-quality water obtained with a Milli-Q water purification system (Millipore, Madrid, Spain) was used for mobile phase B. Mobile phases were filtered through a 0.45 μm nylon 4700 membrane (Waters, Mildford, MA, USA). The mobile phase flow was 1 mL/min and the gradient used is shown in Table 1. The injection volume was 10 μL. Compounds were monitored by fluorescence detection with excitation and emission wavelengths of 250 and 395 nm, respectively.

All chemical analyses were performed in triplicate ($n = 3$).

2.3. Microbial analyses

Microbial analyses were performed at days 0, 7, 14, 21, 28, 35, and 42 of storage.

Mesophilic counts were performed according to the method given in the UNE-EN ISO 4833:2003 standard (AENOR, 2003). Moulds and yeasts were determined following the method described by Harrigan and McCance (1979). *Enterobacteriaceae*, *Staphylococcus aureus*, sulphite-reducing *Clostridium*, *Salmonella* spp., and *Streptococcus faecalis* were enumerated according to the methods described by Pascual and Calderón (2000). H_2S-producing bacteria counts were made by the method described by Lougovois, Kyranas, and Kyrana (2003).

All culture media were provided by Scharlau Chemie, S.A. (Barcelona, Spain). All analyses were performed in duplicate and the results were expressed as log CFU/g.

2.4. Sensory analysis

The sensory evaluation was performed by a panel of 5 assessors (3 females and 2 males) selected from staff of the Department of Food Technology. All of the assessors were familiar with fish quality

Table 1
Elution program for HPLC analysis. A: Methanol LC-grade; B: 20 mmol/L sodium acetate in ultra-high-quality water.

Time (min)	Mobile phase A (%)	Mobile phase B (%)
0	25	75
2	25	75
17	70	30
18	100	0
20	100	0
22	25	75
25	25	75

assessment because had previously taken part in sensory evaluation of different kinds of fish products. Training sessions were held according to the UNE 87024-1 standard (AENOR, 1995). The attributes that best define the sensory quality of smoked sea bass were determined in these sessions. The score sheet consisted of 8-cm hedonic line scales with three-anchor points (very unpleasant, neutral, very pleasant). In each session four samples were evaluated (Table 2). One sample being salted with 100% NaCl (sample Na at day 0); another sample with 50% NaCl-50% KCl (sample Na:K at day 0). The second two samples were smoked and salted with both types of salts, packaged in vacuum, and stored at 4 °C. In the first session they were given 14 days of storage (sample Na and sample Na:K at day 14); and in the second session they were given 28 days of storage (sample Na and sample Na:K at day 28).

Each sample was coded with a 3-digit random number and served to the assessors individually wrapped in aluminium foil. Smoked sea bass was assessed by appearance, fish odour, smoke odour, texture (to the touch), taste, texture (in mouth), and global acceptance. Panellists evaluated all samples in duplicate.

2.5. Statistical analysis

Statistical treatment of the data was performed using the Statgraphics Plus software version 5.1 (Manugistics, Rockville, MD, USA). An analysis of variance (One-Way ANOVA) was conducted for each parameter evaluated to test if there were significant differences between samples. All the chemical, microbiological, and sensory parameters were considered as dependent variables in these analyses. The type of salt (Na or Na:K), packaging procedure (air, vacuum, or modified atmosphere), or time of storage were the factors in these analyses; except for sensory parameters where the factor 'packaging procedure' was not evaluated, since these samples were just vacuum packaged. The LSD procedure (least significant difference) was used to test for differences between averages at the 5% significance level.

3. Results and discussion

3.1. Chemical assessment

Changes in TVB-N for the different samples of smoked sea bass are shown in Fig. 2(a). This parameter is widely used as an indicator of fish spoilage. TVB-N concentration in freshly caught fish is typically between 5 and 20 mg N/100 g flesh (Huss, 1995). In this study the content in the raw material was 9.9 ± 0.5 mg N/100 g. The increase in TVB-N content is related to the activity of spoilage bacteria and endogenous enzymes (Özyurt, Kuley, Özkütük, & Özogul, 2009). There is a great variation in TVB-N acceptability limits in fresh fish. This variation is even higher in processed and semi-processed fish, since the treatment to which the fish are submitted affects the TVB-N contents in the final product. Several authors have reported that this parameter increases with the onset of microbial spoilage (Fernández-Segovia, Escriche, Fuentes, & Serra, 2007; Kykkidou, Giatrakou, Papavergou, Kontominas, & Savvaidis, 2009; Özyurt et al., 2009). In this study the evolution of TVB-N could be related to the growth of mesophilic bacteria, since a similar behaviour was found for these microorganisms (Fig. 3(a). Taking into account this fact, in this study the limit of 35 mg/100 g has been chosen as a possible limit of spoilage (horizontal line in Fig. 2(a)). At the beginning of the storage period, TVB-N values were determined as 12.6 mg/100 g and 11.8 mg/100 g for Na and Na:K samples, respectively. These values are in agreement with those reported in other studies of smoked fish (Gómez-Estaca, Gómez-Guillén, & Montero, 2007; Goulas & Kontominas, 2005). The TVB-N contents significantly increased during storage ($p < 0.001$) in all samples. In general, no significant differences were found depending on the type of salt used (Na or Na:K). At the beginning of the study there were no significant differences in this parameter between the three types of packaging; however, TVB-N values in air packaging increased during storage to a greater extent than in samples packaged in vacuum or MAP. Non-significant differences were found between VP and MAP throughout the study. The smoked sea bass packaged in air exceeded the limit of 35 mg TVB-N/100 g at 30 days of cold storage, while samples packaged in vacuum and in modified atmosphere were below this limit until 42 days of storage.

The evolution of TMA-N in different samples of smoked sea bass is shown in Fig. 2(b). TMA-N concentration in the raw material was 1.75 ± 0.06 mg N/100 g, and the smoking process led to a slight increase in this value. The initial TMA-N contents in both smoked samples were low (2.3 and 2.1 mg/100 g for Na and Na:K samples, respectively), indicating a good quality for the samples recently smoked. The TMA-N contents significantly increased during storage ($p < 0.001$) in all samples, especially from day 35 to the end of storage. In general, all samples exhibited similar values for this parameter, independently of the kind of packaging, except for day 42, when samples stored in air showed values significantly higher ($p < 0.001$). No differences were found depending on the type of salt used. Although TMA-N is believed to be generated by the action of spoilage bacteria, the correlation with bacterial numbers is often not very good (Huss, 1995). In this study, changes in the TMA-N values did not correlate with microbial growth, as occurs in other studies (Özyurt et al., 2009).

To evaluate the degree of lipid oxidation, the thiobarbituric acid index was determined. The evolution of TBA is shown in Fig. 2(c). Values of samples at day 0 were similar to those reported for other smoked fish species (Goulas & Kontominas, 2005). In general, this parameter increased slightly until day 21 of storage, being practically constant during the rest of the study. This variation is indicative of no specific oxidative rancidity trend, as has occurred in other studies (Kykkidou et al., 2009). Different values of acceptability limits have been reported for this index. According to Connell (1995), TBA values of 1–2 mg MDA/kg of fish flesh are usually regarded as the limit beyond which fish will normally develop an objectionable odour. Ruiz-Capillas and Moral (2001) established that the minimum value of TBA index detectable by the panellists was 1.44 mg MDA/kg. However, Nunes et al. (1992) observed that levels of 5–8 mg MDA/kg were generally regarded as the limit of acceptability for fish stored on ice. In this study, no sample reached TBA values higher than 1 mg MDA/kg. Therefore, this parameter cannot be used to assess the spoilage of smoked sea bass under the conditions used in the present work.

Biogenic amines (BAs) are non-volatile compounds, which appear in low concentrations in fresh fish. The accumulation of these compounds is related to the bacterial spoilage of fish, and for this reason they are used as quality markers. In addition, BAs are of interest because of their potential toxicity (Křížek, Vácha, Vorlová, Lukášová, & Cupáková, 2004).

Table 2
Experimental design of the sensory analysis. Na: Smoked sea bass salted with 100% NaCl; Na:K: Smoked sea bass salted with NaCl:KCl (50/50 w/w); V: vacuum packaging.

	Days of storage	Samples evaluated	
		Samples Na in V	Samples Na:K in V
First session	0	X	X
	14	X	X
Second session	0	X	X
	28	X	X

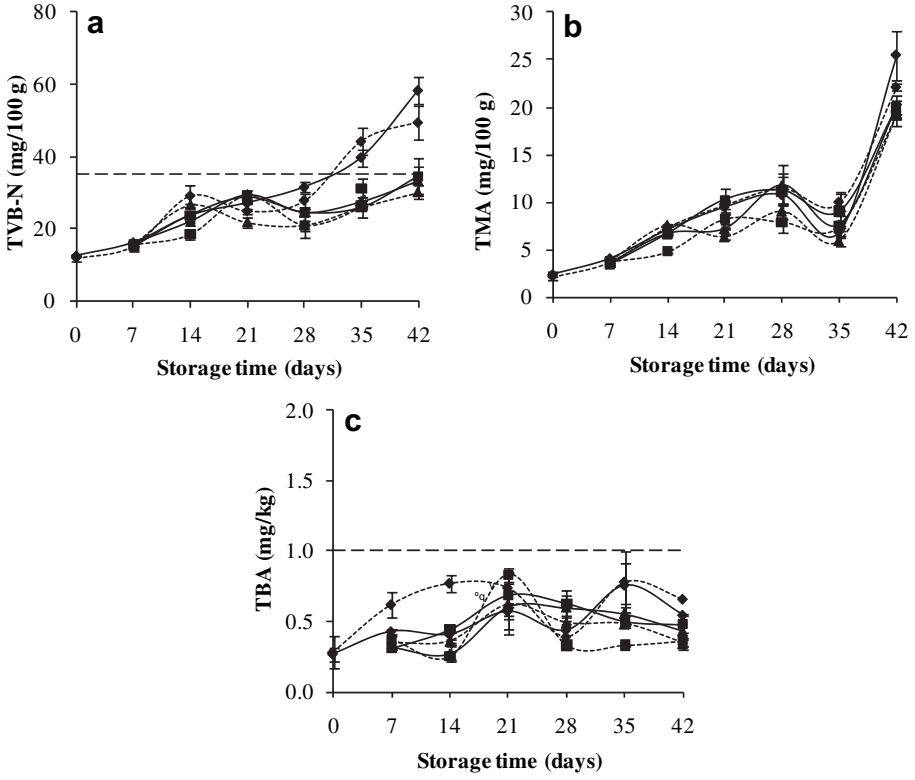

Fig. 2. Evolution of total volatile basic nitrogen (TVB-N) (a), trimethylamine nitrogen (TMA-N) (b), and thiobarbituric acid (TBA) (c), in samples of smoked sea bass salted with 100% NaCl (continuous lines) or 50% NaCl-50% KCl (discontinuous lines), packaged in air (◆), in vacuum (■), or in modified atmosphere (▲), for 42 days storage at 4 °C. Upper areas of horizontal lines are unacceptable in each figure.

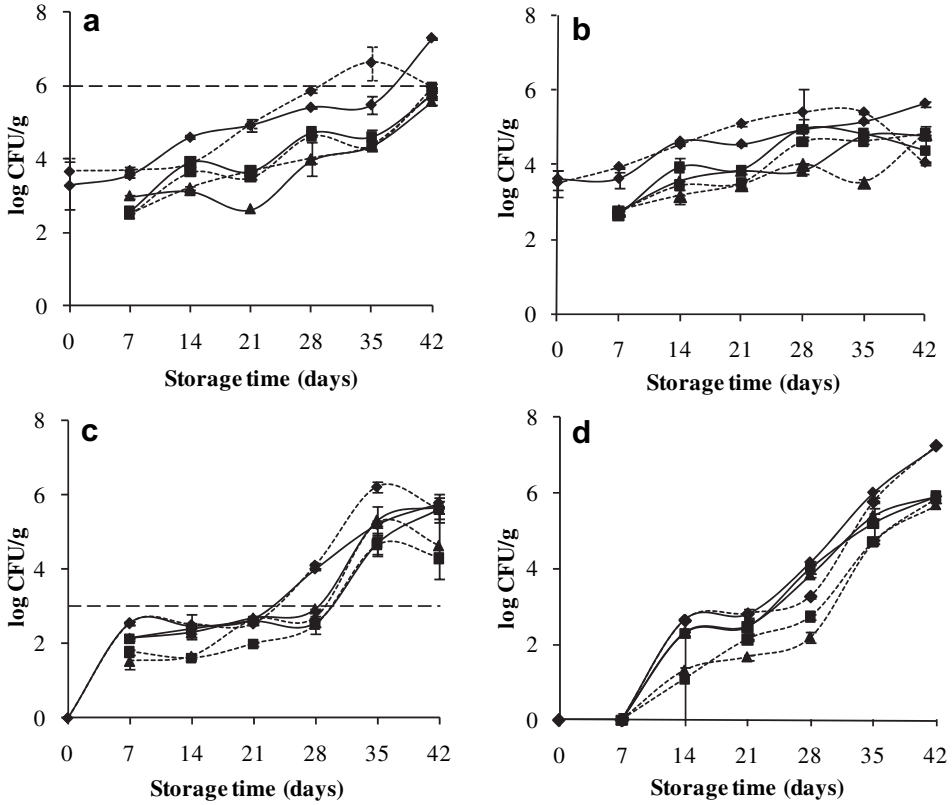

Fig. 3. Evolution of mesophilic bacteria (a), moulds and yeasts (b), *Enterobacteriaceae* (c), and H_2S-producing bacteria (d), in samples of smoked sea bass salted with 100% NaCl (continuous lines) or 50% NaCl-50% KCl (discontinuous lines), packaged in air (◆), in vacuum (■), or in modified atmosphere (▲), for 42 days storage at 4 °C. Upper areas of horizontal lines are unacceptable in each figure.

The concentrations of biogenic amines in smoked sea bass stored in refrigeration are shown in Table 3.

Low contents of histamine (HIS) were found in both samples at the beginning of storage, increasing during storage. The levels of this amine were significantly lower in samples salted with the salt mixture (Na:K) during the study. This could indicate that the partial replacement of NaCl by KCl had an inhibitory effect on HIS formation. No studies on the effect of different types of salts on HIS formation have been found. However, different authors have reported that in salted fish products, the levels of histamine-forming bacteria can change depending on the fish species, processing, handling, salt content, or storage conditions (Křížek et al., 2004; Rodriguez-Jerez, López-Sabater, Roig-Sagues, & Mora-Ventura, 1994; Tsai et al., 2005). In this study, the lower HIS content in Na:K samples could be attributed to the fact that, in the case of using the salt mixture, the amount of salt to be added is higher for reaching the same a_w value (Fuentes et al., 2010). Vacuum and MAP did not show an inhibitory effect on histamine-production.

The FDA (2001) set a guidance level of 50 mg/kg of histamine in *Scombroidae*, since histamine levels in illness-causing fish have usually been above 200 mg/kg, and often above 500 mg/kg. In this study these values were taken as a reference, since no limits for sea bass were found. No sample reached the value of 50 mg/kg of histamine during storage. This could be due to the protective effect of the smoking process. In this sense, several authors have reported that filtered smoke has the ability to negatively affect the microbial growth of *Morganella morganii*, the main bacteria responsible for HYS formation. Moreover, it seems that the smoking process may interfere with the ability of the histamine-forming bacteria to produce HIS (Kristinsson, Danyali, & Ua-Angkoon, 2007).

Table 3
Concentrations of biogenic amines (mg/kg) in samples of smoked sea bass salted with 100% NaCl (Na) or 50% NaCl-50% KCl (Na:K), packaged with air (A), in vacuum (V), or in modified atmosphere (M), for 42 days storage at 4 °C.

	Days of storage	Type of salt and packaging					
		Na			Na:K		
		A	V	M	A	V	M
HIS[a]	0	2.7 ± 0.7			1.2 ± 1.7		
	7	3.0 ± 0.3	3.4 ± 0.6	3.2 ± 0.3	1.0 ± 1.1	1.1 ± 1.9	3.6 ± 1.3
	14	2.2 ± 1.9	4.3 ± 0.4	3.0 ± 0.8	1.1 ± 1.0	1.5 ± 2.5	7.2 ± 1.0
	21	3.8 ± 4.0	8.9 ± 0.8	12.5 ± 3.0	1.0 ± 1.7	2.4 ± 0.4	2.8 ± 0.1
	28	8.9 ± 1.5	41.3 ± 4.3	27.0 ± 0.3	2.1 ± 0.0	2.4 ± 2.1	4.9 ± 0.9
	35	20.9 ± 8.4	36.3 ± 4.5	34.3 ± 2.7	2.1 ± 1.8	3.5 ± 3.1	8.6 ± 2.3
	42	34.0 ± 3.0	35.5 ± 7.2	28.1 ± 1.9	3.5 ± 0.2	9.9 ± 2.3	3.1 ± 1.5
PUT[b]	0	nd			nd		
	7	2.1 ± 0.4	2.7 ± 0.6	3.2 ± 1.3	2.2 ± 0.2	1.9 ± 0.3	2.1 ± 0.8
	14	5.7 ± 1.5	9.8 ± 5.8	20.8 ± 4.3	6.7 ± 1.1	8.2 ± 1.8	8.2 ± 3.1
	21	8.5 ± 2.2	20.0 ± 6.8	59.2 ± 5.2	6.9 ± 0.5	7.4 ± 4.5	13.1 ± 4.4
	28	13.2 ± 4.3	14.0 ± 7.5	11.5 ± 12.6	9.7 ± 4.3	11.2 ± 1.1	10.3 ± 6.7
	35	28.6 ± 11.2	44.0 ± 17.8	25.4 ± 6.7	35.4 ± 17.3	15.6 ± 7.5	13.6 ± 4.0
	42	45.5 ± 12.1	34.2 ± 11.6	38.2 ± 11.8	45.8 ± 10.9	34.9 ± 7.1	22.1 ± 9.7
CAD[c]	0	nd			nd		
	7	0.1 ± 0.0	1.6 ± 2.0	2.0 ± 1.1	0.2 ± 0.3	0.3 ± 0.3	1.5 ± 0.8
	14	3.1 ± 0.7	2.2 ± 1.0	5.3 ± 5.6	1.2 ± 1.0	0.3 ± 0.3	12.5 ± 2.7
	21	0.6 ± 0.5	5.1 ± 4.5	8.7 ± 4.8	0.9 ± 1.4	0.5 ± 0.7	1.4 ± 0.5
	28	4.4 ± 0.5	13.3 ± 10.0	30.0 ± 7.6	0.9 ± 0.1	1.2 ± 0.3	1.8 ± 1.5
	35	20.6 ± 8.5	41.2 ± 20.4	23.2 ± 21.4	2.3 ± 1.1	3.6 ± 0.2	9.2 ± 4.5
	42	25.7 ± 3.6	35.9 ± 8.9	30.4 ± 17.8	3.6 ± 2.9	15.3 ± 1.9	6.0 ± 2.8
TYR[d]	0	1.9 ± 0.8			1.8 ± 1.1		
	7	20.2 ± 16.9	64.99 ± 12.4	59.7 ± 10.5	47.5 ± 44.2	73.0 ± 23.3	66.0 ± 20.4
	14	21.1 ± 19.8	101.29 ± 18.3	136.0 ± 24.2	45.1 ± 29.2	54.1 ± 68.3	157.3 ± 11.1
	21	14.0 ± 8.4	180.09 ± 71.1	245.4 ± 43.0	86.5 ± 62.1	75.2 ± 71.4	104.4 ± 48.1
	28	21.1 ± 4.1	144.69 ± 30.9	170.0 ± 69.9	108.5 ± 17.0	135.8 ± 15.8	168.4 ± 24.4
	35	143.2 ± 56.2	187.79 ± 88.7	187.1 ± 65.7	170.2 ± 39.9	173.1 ± 14.2	200.9 ± 36.7
	42	170.0 ± 13.5	173.99 ± 21.4	221.9 ± 20.6	153.4 ± 35.1	223.1 ± 12.3	236.7 ± 36.2
SPD[e]	0	4.3 ± 0.8			4.0 ± 0.6		
	7	2.8 ± 0.0	4.0 ± 0.6	7.1 ± 2.6	6.7 ± 1.0	3.4 ± 1.0	3.1 ± 1.1
	14	7.1 ± 3.6	5.6 ± 0.4	10.0 ± 8.1	9.9 ± 0.5	7.5 ± 7.3	6.4 ± 4.0
	21	13.2 ± 1.8	11.5 ± 0.4	4.0 ± 0.1	11.9 ± 0.9	3.6 ± 0.5	4.4 ± 0.6
	28	7.7 ± 1.6	5.7 ± 1.5	12.2 ± 7.7	3.8 ± 0.2	4.9 ± 0.8	3.6 ± 0.1
	35	4.1 ± 0.4	3.8 ± 0.8	3.7 ± 0.4	3.5 ± 0.4	4.9 ± 0.1	4.6 ± 0.7
	42	6.1 ± 2.2	8.7 ± 1.9	8.1 ± 3.2	3.4 ± 0.6	4.2 ± 2.2	5.1 ± 1.2
SPN[f]	0	4.3 ± 2.3			3.2 ± 1.1		
	7	1.3 ± 1.1	0.9 ± 1.6	3.1 ± 1.3	9.5 ± 1.4	1.9 ± 3.3	2.8 ± 1.3
	14	2.2 ± 0.3	3.1 ± 1.6	4.9 ± 5.4	0.9 ± 0.5	0.9 ± 0.9	2.6 ± 1.0
	21	0.5 ± 0.3	0.5 ± 0.4	2.6 ± 0.3	1.5 ± 0.3	1.5 ± 1.0	2.5 ± 0.2
	28	1.0 ± 0.7	8.9 ± 5.8	8.3 ± 5.6	0.6 ± 0.5	1.5 ± 0.3	1.0 ± 0.1
	35	2.8 ± 1.3	1.4 ± 1.2	1.0 ± 0.6	1.3 ± 1.4	1.6 ± 0.7	0.9 ± 0.2
	42	2.8 ± 1.0	5.1 ± 2.5	4.1 ± 1.3	1.8 ± 0.9	2.4 ± 1.6	1.1 ± 0.4

[a] HIS: Histamine.
[b] PUT: Putrescine.
[c] CAD: Cadaverine.
[d] TYR: Tyramine.
[e] SPD: Spermidine.
[f] SPN: Spermine.

Although toxicity is most closely linked to the development of histamine, there are indications that other amines, such as putrescine (PUT) and cadaverine (CAD) can cause illness, even in the absence of histamine formation (FDA, 2001). In this work, putrescine or cadaverine were not found in the samples recently smoked, but their levels increased during storage (Table 3). In general, no effect of the type of packaging was found on the evolution of PUT or CAD. However, the kind of salt significantly affected the levels of these amines, being lower in samples with sodium replacement, as occurred for histamine. The similarity between PUT and CAD evolutions has been observed in other studies (Gökoglu, Yerlikaya, & Cengiz, 2003).

Tryptamine (TRP) was not detected in any sample during the storage time. This could be due to the fact that low temperatures inhibit or delay the formation of this amine, as reported by Pons-Sánchez-Cascado, Vidal-Carou, Mariné-Font and Venciana-Nogués (2006). Low levels of tyramine (TYR) were found at the beginning of storage. A large increase was observed during storage, being the BA which showed higher levels during the studied period, as was observed in other studies of fish storage (Pons-Sánchez-Cascado, Vidal-Carou, Mariné-Font et al., 2006; Pons-Sánchez-Cascado, Vidal-Carou, Nunes, & Venciana-Nogués, 2006). The levels of this amine were significantly lower during the whole study in samples salted with NaCl packaged in air ($p < 0.001$). Spermidine (SPD) and spermine (SPN) were found to show a similar evolution during storage. The concentrations of both amines fluctuated during storage. This could be due to the fact that SPD and SPN occur naturally in food and their formation is not related to bacterial spoilage (Özyurt et al., 2009; Pons-Sánchez-Cascado, Vidal-Carou, Nunes et al., 2006). In general, no significant differences in both amines were found depending on the kind of packaging or salt used.

3.2. Microbial assessment

No *S. aureus*, sulphite-reducing *Clostridium*, *Salmonella*, or *S. faecalis* were isolated from any of the analyzed samples.

Counts of mesophilic, moulds and yeasts, *Enterobacteriaceae*, and H_2S-producing bacteria found in smoked sea bass during the course of 42 days of storage are shown in Fig. 3. Initial counts of all microorganisms were low. In general, the highest microbial growth was found in samples packaged in air, independently of the type of salt used. MAP was the most effective packaging, although samples in VP showed counts very close to those of MAP. These results agree with other authors (Fernández-Segovia et al., 2007; Sivertsvik et al., 2002) who reported that packaging of fish products under modified atmospheres increases shelf life compared with those packaged in air; but confers little or no additional shelf life when compared with vacuum packaging.

The low levels of mesophilic bacteria at the beginning of storage indicates good fish quality (Fig. 3(a)). A large increase was observed during storage. In this work, 10^6 CFU/g of mesophilic bacteria has been used as the limit for the evaluation of microbial spoilage. This figure is the Spanish legal limit for smoked fish (MSC, 1991). The smoked samples packaged in air were close to this limit at day 28 of storage. Mesophilic growth was lower in VP and MAP and did not reach the upper limit; however, by the end of storage, growth was well over 10^5 CFU/g and the product was close to spoilage. VP and MAP achieved a shelf life extension of 10 days when compared with packaging in air. This is in agreement with other research findings (Kykkidou et al., 2009). In general, no differences were found between salts.

Mould and yeast counts were higher in samples packaged in air during the first weeks of the study (Fig. 3(b)). However, these differences decreased during storage, and all samples reached similar values at the end of the study. Truelstrup-Hansen, Gill, Drewes-Rontved, and Huss (1996) mentioned the minor contribution of moulds and yeasts to the spoilage of smoked fish products, and this may explain why a large growth of these microorganisms was not observed in any of the samples during storage. No differences that depended on the type of salt used were observed, as occurs in mesophilic counts.

No *Enterobacteriaceae* were found in samples recently smoked, indicating a good level of hygiene during the handling and processing. There was a large increase during storage (Fig. 3(c)). No differences in the evolution of this microorganism were found between samples salted with Na or Na:K in samples packaged in air. However, for MAP and especially for VP, counts in Na:K samples were lower. Counts of these microorganisms stood under the Spanish legal limit of 10^3 CFU/g (MSC, 1991) until day 21 of storage in samples packaged in air. Samples in VP and MAP did not reach this limit until day 35, although counts were very close to 10^3 CFU/g at day 28.

The evolution of H_2S-producing bacteria is shown in Fig. 3(d). No growth of these microorganisms was observed in any sample during the first week of storage. In general, counts in Na samples were higher than those in Na:K samples, except for day 42 of storage. These bacteria reached high levels at the end of the study period, especially samples packaged in air. Bacteria belonging to this group, mainly *Shewanella putrefaciens*, have been identified as the main responsible of fish spoilage in ice or cold storage (Dalgaard, Gram, & Huss, 1993). There is great variability in the acceptability limits found for H_2S-producing bacteria. In this sense, Kyrana and Lougovois (2002) detected counts of 4.5×10^4 CFU/g in sea bass stored on melting ice, when fish were sensory rejected. Lougovois et al. (2003) stated that an average level of 10^6 CFU/g of H_2S-producing bacteria would be indicative of marginal quality gilthead sea bream. In this study, the time of sensory rejection of samples in VP was less than 28 days of storage, corresponding to levels of H_2S-producing bacteria lower than 10^3 CFU/g. This low bacterial load could mean that the early loss of odour and other sensory attributes resulted primarily from autolytic reactions.

3.3. Sensory assessment

The results of sensory evaluation of smoked sea bass salted with both types of salts and vacuum packaged during 28 days of storage are presented in Fig. 4. VP was the only type of packaging studied in this part of the work, since it was effective in the extension of shelf life compared with air packaging. MAP was not included in this analysis because no important differences in the studied chemical parameters

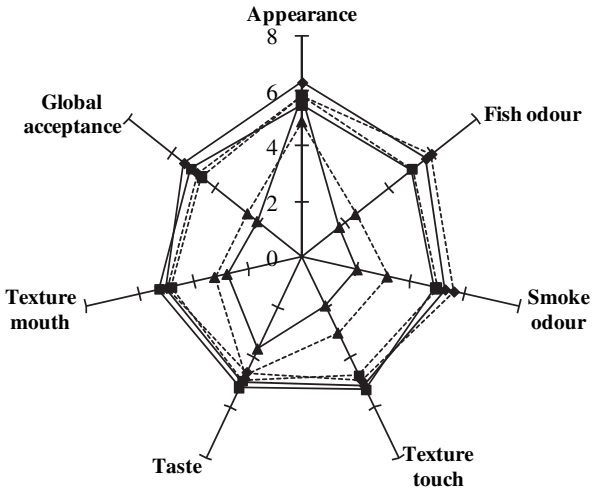

Fig. 4. Score average for the different attributes evaluated in samples of smoked sea bass salted with 100% NaCl (continuous lines) or 50% NaCl-50% KCl (discontinuous lines), packaged in vacuum stored for 0 (◆), 14 (■), or 28 (▲) days at 4 °C.

or microbial growth were found between samples in VP and those in MAP, and also because of the lower cost of vacuum packaging.

In general, no significant differences were found for any of the evaluated attributes depending on the type of salt. This means that the partial replacement of 50% sodium by potassium did not affect the sensory quality of the product. The highest scores were given for recently smoked samples, although the differences with the attribute scores at day 14 of storage were not significant. However, samples stored during 28 days had lower scores in all attributes, except for appearance and taste. The low scores of these samples (lower than 4) means that panellists did not consider acceptable the quality of smoked sea bass salted with 100% NaCl or 50% NaCl-50% KCl after 28 days in refrigeration. According to these results, the shelf life for samples packaged in vacuum is lower than 28 days. This period is in agreement with the results found in the microbial analysis for *Enterobacteriaceae* counts.

4. Conclusions

In general, partial sodium replacement did not affect chemical, microbiological or sensory quality. However, partial replacement of NaCl by KCl inhibited histamine, putrescine, and cadaverine formation. Further studies on the effect of the substitution of NaCl by KCl on histamine-forming bacteria would be interesting. The combination of 50% NaCl with 50% KCl is a good alternative to obtain a smoked sea bass product with low sodium content with the same features than the traditional product.

Vacuum and modified atmosphere packaging reduced microbial growth and the production of volatile basic N compounds, in comparison with air packaging. However, this reduction was not high enough to prevent microbial spoilage of the product, and high levels of TVB-N were observed throughout the storage period. Since no important differences between VP and MAP were found, the use of vacuum would be more suitable due to its lower cost. The shelf life of smoked samples (with or without NaCl partial replacement) is under 28 days. The application of other preservation treatments, such as additive incorporation, high pressures, or hurdle technology, is necessary to increase shelf life, and consequently, the marketing period of smoked sea bass.

Acknowledgements

The revision of this paper was funded by the Universidad Politécnica de Valencia, Spain.

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