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Effect of salting and smoking-method on the keeping quality of chub mackerel (*Scomber japonicus*): biochemical and sensory attributes

Antonios E. Goulas *, Michael G. Kontominas

Department of Chemistry, Laboratory of Food Chemistry and Technology, University of Ioannina, GR-45110 Ioannina, Greece

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Abstract

The effect of salting and smoking method on the keeping quality of chub mackerel was studied over a period of 30 days. Quality assessment was based on sensory analysis and biochemical indices determination. The effect of salting on the preservation of non-smoked chub mackerel packaged and stored under the same conditions as the smoked samples was also studied. The two smoking methods applied, resulted in a clear preservative effect on fish samples. Total volatile basic nitrogen and trimethylamine nitrogen values of salted and smoked samples remained practically constant during the 30 day storage period. Salting had a noticeable preservative effect but lower than the combined effect of salting and smoking. 2-Thiobarbituric acid (TBA)-reactive substances of salted and smoked samples increased during the storage period but remained at low levels after 30 days of storage in contrast to TBA values of non-smoked samples. Relatively constant sensory characteristics were observed for salted and smoked samples during the 30 day storage period while slight differences were observed between two smoking methods mainly differences in texture. A significant reduction in the sensory scores was recorded for non-smoked samples during storage; which was higher for unsalted versus salted samples.

Keywords: Hot-smoked chub mackerel; Salting; Vacuum-packaging; Shelf-life; Biochemical analysis; Sensory assessment

1. Introduction

Smoke curing is a traditional fish preservation method of considerable economic importance worldwide. Smoke, is produced by the process of incomplete combustion of wood. It consists of numerous individual components namely: aldehydes, ketones, alcoholes, acids, hydrocarbons, esters, phenols, ethers, etc. (Doe, 1998; Guillen & Errecalde, 2002). These components are transfered to the smoked goods by deposition on their surface and subsequent penetration into their flesh (Doe, 1998).

Smoking imparts a characteristic flavour and colour to the fish. In addition, smoking increases the shelf-life of fish as a result of the combined effects of dehydration, antimicrobial and antioxidant activity of several of the smoke constituents mainly: formaldehyde, carboxylic acids, phenols (Doe, 1998; Horner, 1997; Leroi & Joffraud, 2000a; Rorvik, 2000). An additional preservative effect is owed to salting which comprises the first step of the fish smoking process.

The preservative effect of salting is mainly due to the decrease in water activity (a_w) and thus prevention of growth of many spoilage microorganisms along with the formation of a more membranous surface which further inhibits the growth of microorganisms (Horner, 1997; Leroi & Joffraud, 2000a; Rorvik, 2000).

* Corresponding author. Tel.: +32 6510 97390; fax: +32 6510 98795.
E-mail address: aegoulas@cc.uoi.gr (A.E. Goulas).

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Moreover, chloride ions are toxic for some microorganisms (Leroi, Joffraud, & Chevalier, 2000b).

There are three methods used to smoke fish: the traditional method by combustion, at either low temperature (cold smoking $\leqslant 30$ °C) or high temperature (hot smoking $\geqslant 60$ °C); use of a high voltage electrostatic field which accelerates smoke deposition; and use of liquid smoke which lowers the content of polynuclear aromatic hydrocarbons (potently carcinogenic compounds) in liquid smoked fish (Doe, 1998; Duffes, 1999; Espe, Nortvedt, Lie, & Hafsteinsson, 2002; Hattula, Elfving, Mroueh, & Luoma, 2001; Sigurgisladottir, Sigurdardottir, Torrissen, Vallet, & Hafsteinsson, 2000).

Production of smoked fish in Greece has rapidly increased over the past few years; from 1010 tons/year in 1996 to 2350 tons/year in 1999 (Anonymous, 2000). Among smoked species the hot-smoked products are the most widely consumed in Greece.

Hot-smoking is a pasteurizing process, the preservative effect of which depends on the composition and preparation of raw material, temperature, relative humidity, density and composition of the smoke as well as the smoking time (Doe, 1998; Kolodziejska, Niecikowska, Januszewska, & Sikorski, 2002).

Chub mackerel (*Scomber japonicus*) is a common fish in the Mediterranean sea. Statistical data show that 3041 tons were caught in Greece in 1999 corresponding to 6% of the harvesting of all fish species (Anonymous, 2000). Chub mackerel is usually sold as a raw product. The application of hot smoking for the extension of the shelf-life is a process of interest, given that chub mackerel is generally a fatty fish which spoils easily.

The objectives of the present work were to study the effects of salting and different hot smoking procedures on chub mackerel fillet quality as assessed by sensory analysis and determination of biochemical indices.

2. Materials and methods

2.1. Fish samples

Fresh chub mackerel was purchased from local distributors approximately 12 h after harvesting and transferred within one hour to the fish processing plant (TSIALIOS S.A., Ioannina, Greece) in sealed foamed polystyrene boxes containing ice. The average weight of the whole fish was 500 g. Average length was 25 cm. Fishing ground was the Ionian Sea, Greece. Catching method used was surrounding nets fishing. The fish were subsequently headed and filleted. The fillets were separated into four groups; two of which were salted and smoked according to the two hot-smoking methods described in Fig. 1. The two other fillet groups were used to study the effect of salting on the shelf-life and quality of non-smoked mackerel fillets under refrigeration. Control samples included unsalted non-smoked, vacuum packed chub mackerel (group 4). For each fish the fillet from the right was utilized for smoking method 1 and the fillet from the left was used for smoking method 2. The same was done for the non-smoked salted and unsalted chub mackerel samples.

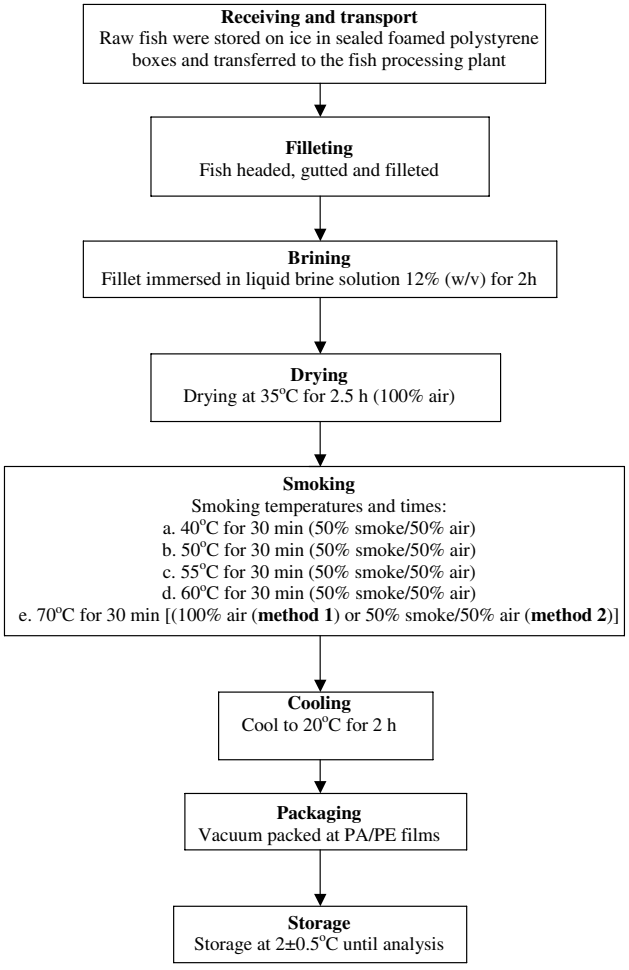

Fig. 1. Flow diagram of the production of hot-smoked chub mackerel.

2.2. Salting and smoking of samples

Chub mackerel fillets were immersed in a brine containing 120 g/L NaCl at 8 °C for 2 h with a fish:brine ratio of 1:1 (w/v). Then the fillets were dried and smoked in a conventional smoking facility equipped with an automatic control for temperature, humidity and density of wood smoke. After chilling the smoked products were vacuum-packed in Polyamide/Low density Polyethylene (PA/LDPE) (20/80) μm bags (VER PACK S.A., Thessaloniki, Greece) using a BOSS model N48 vacuum sealer. The oxygen permeability of bags was 82 $cm^3\ m^{-2}\ day^{-1}\ atm^{-1}$ at 75% relative humidity

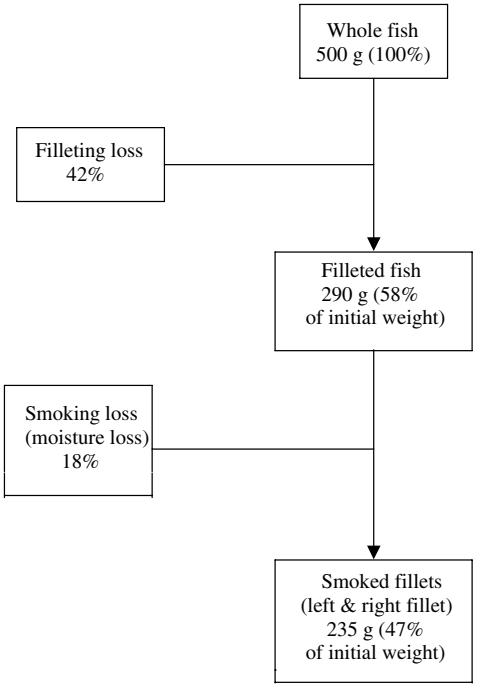

Fig. 2. Yield and loss during production of hot-smoked chub mackerel fillets ($n = 2 \times 3$).

(RH), 23 °C and the water vapour permeability of bags was 2.7 g m^{-2} day^{-1} at 100% RH, 23 °C. After packing, the fish were transferred to the laboratory in sealed foamed polystyrene boxes containing ice and stored at 2–3 °C for a period of 30 days. Sampling was carried out at predetermined time intervals namely: 0, 1, 6, 12, 18, 24 and 30 days. At each sampling day 3 fish were randomly chosen for analysis. The experiment was duplicated for each fish at different time periods ($3 \times 2 = 6$ samples). Fig. 1 presents detailed steps of the production procedure while Fig. 2 presents the yield and losses during the production of smoked chub mackerel.

2.3. Non-smoked samples

One group of chub mackerel fillets were brined after immersion in the same as above reported brine for 2 h at 8 °C. Another group of fillets was stored for 2 h at 8 °C without any treatment and was used as the control sample. Subsequently, the two groups of fillets were vacuum-packed in the same PA/LDPE bags as above reported and stored at 2–3 °C for the same as above period.

2.4. Moisture content

Moisture content was determined by oven drying of 5 g of fish fillet at 105 °C until a constant weight was obtained (AOAC, Official method 985.14, 1995).

2.5. Salt content

Salt content was determined using the method of AOAC (AOAC, Official method 937.09, 1995).

2.6. Determination of pH

A 10 g sample of the fish flesh was homogenised in 100 ml of distilled water and the mixture was filtered. The pH of filtrate was measured using a CRISON model 507 pH-meter at ambient temperature (CRISON Instruments, Barcelona, Spain).

2.7. Biochemical analysis

2.7.1. Determination of total volatile basic nitrogen

A 10 g sample of fish flesh was mixed with 50 ml of distilled water using a Moulinex mixer. The mixture was quantitatively transferred with 200 ml of distilled water into a 500 ml round bottom flask and was distilled after the addition of 2 g of MgO and one drop of silicone to prevent foaming. A 250 ml Erlenmeyer flask containing 25 ml of 3% aqueous solution of boric acid, 0.04 ml of methyl red and methylene blue mixed indicators for the titration of ammonia was used as the distillate receiver. Distillation was continued until a final volume of 125 ml of distillate was obtained. The boric acid solution turned green when made alkaline by the distilled total volatile basic nitrogen (TVBN). This was titrated with an aqueous 0.1 N hydrochloric acid solution. Complete neutralization was achieved when the color of the distillate turned pink upon addition of a further drop of hydrochloric acid. The quantity of TVBN in mg/100 g of fish flesh was calculated from the volume (V) of hydrochloric acid added and its concentration (C) as follows:

$$\% \text{ mg TVBN} = (V \times C \times 14 \times 100)/10.$$

2.7.2. Determination of trimethylamine nitrogen

Trimethylamine nitrogen (TMAN) was determined using the above mentioned TVBN method after appropriate modification: formaldehyde was used to block the primary and secondary amines (Malle & Tao, 1987). A 100 g sample of fish flesh was mixed with 100 ml of distilled water. The mixture was quantitatively transferred with 30 ml of distilled water into a round bottom flask and was distilled after the addition of 20 g of MgO, 100 ml of 20% aqueous solution of formaldehyde (HCHO) and five drops of silicone anti-foaming agent. The same procedure was then used as for TVBN determination and 125 ml of distillate were collected. The distillate was titrated using aqueous 0.05 N hydrochloric acid solution. The amount of TMAN in mg/100 g of fish flesh was calculated from the volume (V) of hydrochloric acid added and its concentration (C) as follows:

$$\% \text{ mg TMAN} = V \times C \times 14.$$

2.7.3. Determination of 2-thiobarbituric acid

The method is based on the spectrophotometric quantitation of the pink complex formed after reaction of one molecule of malondialdehyde (MDA) with two molecules of 2-thiobarbituric acid (TBA).

2.7.3.1. Preparation of TBA solution. The TBA solution was prepared by weighing 0.67 g of TBA and transferring in a 100 ml beaker with 90 ml distilled water. The beaker was placed in a water bath (80 °C) until complete dissolution. The solution was then quantitatively transferred to a 100 ml volumetric flask and the volume completed with distilled water. The above sample (25 ml) were transferred into a 250 ml Erlenmeyer flask with a glass stopper and was mixed with 25 ml of 100% glacial acetic acid.

2.7.3.2. Determination of TBA. Fish flesh (50 g) was homogenized after the addition of 5 ml butylated hydroxytoluene (BHT, 1 g l^{-1}) to prevent autoxidation. The homogenized fish flesh (6 g) was weighed and transferred in a 250 ml Erlenmeyer flask fitted with a glass stopper containing 12 ml of carbon tetrachloride and 40 ml of TBA solution. The flask was stoppered and shaken vigorously for 10 s and the content was centrifuged at 1350*g* for 7 min and filtered through a Whatman No. 1 filter paper and transferred into a screw-cap test tube. The tube was placed in a water bath (80 °C) for 30 min until the pink color developed completely. The test tube was then cooled and the optical density was determined at 532 nm on a SECOMAM ANTHELIE Spectrophotometer Type 70ST0375 (Secomam, France) using distilled water as a control. TBA values were expressed as mg of malondialdehyde (MDA)/kg of sample. The concentration of malondialdehyde was calculated from a standard curve using solutions of the MDA precursor (same molecular weight) 1,1,3,3-tetraethoxy-propane (TEP) into distilled water after the addition of a quantity of TBA solution.

2.8. Sensory evaluation

2.8.1. Smoked samples

The sensory quality of smoked chub mackerel fillets was evaluated at each sampling time (day 0, 6, 12, 18, 24, 30) by a seven member trained panel. Prior to sensory assessment samples were removed from the refrigerator and held for 30 min at room temperature. Samples were then cut in ca. 30 g pieces and presented to each panelist in plastic cups covered with a lid in random order. Panelists were asked to score odour, taste, flesh colour and texture of fillets using a 0–10 acceptability scale where a score of 10 was defined as excellent. For each sensory attribute a score of 6 (recorded by at least of 50% of the judges) was considered to be the lower limit of acceptability, implying that shelf life was terminated when this score was obtained.

2.8.2. Non-smoked samples

For the non-smoked samples panelists were asked to score the odour, flesh colour and texture of salted and unsalted fillets using the same as above referred 0–10 acceptability scale. Raw chub mackerel fillets were blast frozen at −30 °C throughout the experiment to serve as control samples during sensory evaluation of non-smoked samples. Evaluation of frozen samples was carried out after thawing at room temperature.

2.9. Statistical analysis

Experiments were replicated twice on different occasions with different fish samples. All analyses were run in triplicate for each replicate. Results are reported as mean values of six determinations ± standard deviation (SD). Data were subjected to analysis of variance (ANOVA). The last significant difference (LSD) procedure was used to test for difference between means (significance was defined at $p < 0.05$).

3. Results and discussion

3.1. Determination of moisture content

Moisture content of chub mackerel fillets are given in Table 1. Mean moisture values of 59.0% and 58.1% were recorded for samples smoked by method 1 and 2, respectively. The corresponding mean values for unsalted non-smoked (USNS) and salted non-smoked (SNS) samples were 75.2% and 74.9%, respectively.

There were no statistically significant differences ($p < 0.05$) in moisture content between fillets smoked by the two different methods or between USNS and SNS samples.

The smoking process resulted in a significant decrease ($p < 0.05$) in moisture content of chub mackerel samples. This decrease corresponds to a 21.2% and 22.4% loss of the moisture content of the raw samples, respectively.

Industrial specifications for "smoked finished products" generally recommend a water content in the fish flesh of less than 65% (Cardinal et al., 2001). This is in agreement with our values of 59.0% and 58.1%, respectively. These values are in complete agreement with those of Kolodziejska et al. (2002), who reported that the mean moisture content of smoked mackerel was 56.7%.

Table 1
Moisture content[A] of vacuum packaged chub mackerel stored at 2 ± 0.5 °C as a function of storage time

Storage time[B] (days)	Salted, smoked (method 1)	Salted, smoked (method 2)	Salted, non-smoked	Unsalted, non-smoked
0	75.30 ± 0.86[a]	75.30 ± 0.86[a]	75.30 ± 0.86[a]	75.30 ± 0.86[a]
1	56.81 ± 0.87[a]	56.67 ± 0.28[a]	75.04 ± 0.35[b]	75.36 ± 0.51[b]
6	59.24 ± 0.26[a]	59.06 ± 0.50[a]	74.73 ± 0.95[b]	75.20 ± 0.82[b]
12	56.33 ± 0.41[a]	56.18 ± 0.28[a]	75.32 ± 1.39[b]	74.75 ± 0.90[b]
18	61.50 ± 0.96[a]	59.44 ± 1.28[a]	74.52 ± 0.64[b]	75.13 ± 0.37[b]
24	60.77 ± 0.91[a]	59.71 ± 0.48[a]	74.28 ± 0.52[b]	74.95 ± 0.58[b]
30	59.35 ± 1.38[a]	57.34 ± 0.79[a]	75.51 ± 1.41[b]	75.80 ± 0.46[b]

[a,b] Values in the same line followed by different letter are significantly different ($p < 0.05$).
[A] Values represent the mean of six determinations ($n = 2 \times 3$) ± SD.
[B] Values on day 0 correspond to unsalted, non-smoked product.

3.2. Determination of salt content

Salt content of chub mackerel fillets are given in Table 2. A statistically significant ($p < 0.05$) lower salt content was found in the non-smoked versus the smoked samples. Mean values of salt content obtained in the fish muscle were as follows: 3.6% for SNS samples and 5.8% and 5.7% for the smoked (methods 1 and 2) samples, respectively. According to the literature (Horner, 1997) most of the microorganisms normally associated with fish spoilage are halophobic and will not grow in salt concentrations exceeding 5%. Similar results for the preservative effect of salt have been reported by Kolodziejska et al. (2002), for hot smoked mackerel.

The increase of 2.1–2.2% in the salt content of smoked samples is due to partial dehydration during the smoking process and subsequent changes in the wet weight of the fillets. There are no statistically significant differences ($p < 0.05$) in the salt content of fillets smoked by the two smoking methods. Specific brining conditions used in our study for the salting of fillets is recommended for fatty fish such as chub mackerel (Doe, 1998).

Even though salting effectively prevents the growth of both spoilage and pathogenic bacteria (Doe, 1998; Leroi et al., 2000b); it has been reported that salt content in fish muscle enhances oxidation of the highly unsaturated lipids (Aubourg & Ugliano, 2002).

3.3. Determination of pH

The changes in the pH of chub mackerel fillets are given in Table 3. The initial pH of untreated fillets was 6.12. This pH value is in agreement with that of Metin, Erkan, Varlik, and Aran (2001), who reported a pH value of 6.06 for fresh chub mackerel before treatment.

The pH remained constant during storage of salted and smoked samples but it increased progressively during the storage of the non-smoked samples. In SNS samples pH increased from 6.17 to 6.57 while in USNS samples, pH increased from 6.33 to 6.78. The increase of pH may be attributed to the production of volatile basic components, such as ammonia, trimethylamine etc. by fish spoiling bacteria (Hyytia, Hielm, Mokkila, Kinnunen, & Korkeala, 1999; Reddy et al., 1997; Ruiz-Capillas & Moral, 2001a). No significant differences ($p < 0.05$) were observed between fillet samples smoked by the two different methods. The significantly lower ($p < 0.05$) pH of smoked versus non-smoked samples (approximately 0.5 unit at the end of storage) enhances microbial inhibition and contributes to the extension of product shelf life. Similar differences have been reported by Leroi and Joffraud (2000a) and Doe (1998) and may be attributed to the lower moisture content of smoked samples and to the salting. Leroi and Joffraud (2000a) reported that salt had a highly significant linear decreasing effect on the pH, which remained

Table 2
NaCl content (g NaCl/100 g of sample)[A] in chub mackerel stored at 2 ± 0.5 °C as a function of storage time

Storage time (days)	Salted, smoked (method 1)	Salted, smoked (method 2)	Salted, non-smoked
0	0.1 ± 0.04[a]	0.1 ± 0.04[a]	0.1 ± 0.04[a]
1	6.2 ± 0.5[a]	6.4 ± 0.9[a]	3.8 ± 0.4[b]
6	6.1 ± 1.1[a]	5.6 ± 0.7[a]	3.7 ± 0.6[b]
12	5.7 ± 0.9[a]	5.4 ± 0.5[a]	3.5 ± 0.9[b]
18	5.3 ± 0.3[a]	5.5 ± 0.2[a]	3.4 ± 0.8[b]
24	5.5 ± 0.7[a]	5.9 ± 0.6[a]	3.8 ± 0.3[b]
30	5.4 ± 0.5[a]	6.2 ± 0.3[a]	3.4 ± 0.6[b]

[a,b] Values in the same line followed by different letter are significantly different ($p < 0.05$).
[A] Values represent the mean of six determinations ($n = 2 \times 3$) ± SD.

Table 3
pH values[A] of chub mackerel stored at 2 ± 0.5 °C as a function of storage time

Storage time[B] (days)	Salted, smoked (method 1)	Salted, smoked (method 2)	Salted, non-smoked	Unsalted, non-smoked
0	6.12 ± 0.09[a]	6.12 ± 0.09[a]	6.12 ± 0.09[a]	6.12 ± 0.09[a]
1	6.00 ± 0.06[a]	5.93 ± 0.08[a]	6.17 ± 0.10[b]	6.33 ± 0.05[c]
6	5.95 ± 0.10[a]	6.00 ± 0.04[a]	6.19 ± 0.04[b]	6.36 ± 0.09[c]
12	6.01 ± 0.03[a]	6.05 ± 0.11[a]	6.38 ± 0.12[b]	6.44 ± 0.06[b]
18	6.04 ± 0.12[a]	6.02 ± 0.09[a]	6.40 ± 0.04[b]	6.53 ± 0.05[c]
24	6.03 ± 0.05[a]	6.03 ± 0.07[a]	6.42 ± 0.15[b]	6.67 ± 0.06[c]
30	6.10 ± 0.11[a]	6.08 ± 0.13[a]	6.57 ± 0.10[b]	6.78 ± 0.07[c]

[a–c] Values in the same line followed by different letter are significantly different ($p < 0.05$).
[A] Values represent the mean of six determinations ($n = 2 \times 3$) ± SD.
[B] Values on day 0 correspond to unsalted, non-smoked product.

constant during smoked salmon storage. The pH decrease in fish flesh by the addition of salt is explained by the increase of the ionic strength of the solution inside of the cells (Leroi & Joffraud, 2000a). This is confirmed by our data for non-smoked (salted and unsalted) samples, where pH decreased from 6.33 to 6.17 when salt was added to fish fillets (day one of storage). This difference remained statistically significant ($p < 0.05$) throughout the entire storage period.

3.4. Determination of total volatile basic nitrogen

Total volatile basic nitrogen (TVBN) values for chub mackerel are presented in Table 4. The initial TVBN values of untreated fillet samples on day 0 (10.93 mg N/100 g) is indicative of freshness of raw fish material and is in agreement with the relatively low initial TMAN content. This value is in good agreement with that of Metin et al. (2001), who reported that the initial TVBN content in raw chub mackerel was 9.96 mg N/100 g. Similar TVBN value was reported for fresh hake: 10.44 mg N/100 g (Ruiz-Capillas, Morales, & Moral, 2001b).

As results show, the TVBN level increased gradually with time of storage, for both non-smoked samples either with or without salting. TVBN increase is expected because it is related to bacterial spoilage (Connell, 1990). This increase was significantly lower ($p < 0.05$) in the SNS samples than in the USNS samples and can be attributed to the preservative effect of salt (Hansen, Gill, & Huss, 1995; Kolodziejska et al., 2002; Leroi et al., 2000b).

In contrast, TVBN values remained practically constant in smoked samples independent of storage time. This is associated with lower moisture content, higher salt level and deposition of antimicrobial smoke constituents (e.g. phenols, formaldehyde, etc.) on smoked samples than corresponding non-smoked samples. This observation is in agreement with the results of sensory assessment.

Another observation is that the initial TVBN values of smoked samples (20.94 and 20.18 mg N/100 g) are significantly higher ($p < 0.05$) than the corresponding values of non-smoked samples (11.28 and 11.15 mg N/100 g). This difference is due to partial dehydration of smoked samples (Table 1) and subsequent concentration of TVBN constituents. As time of storage progressed the TVBN values of non-smoked samples increased steadily and surpassed the corresponding TVBN values of smoked samples. The above results are in agreement with those reported by Plahar, Nerquaye-Tetteh, and Annan (1999) for smoked sardinella and anchovies. This crossover in TVBN values between smoked and non-smoked samples was observed between day 12–18 of storage and continued until the end of the storage period. After 30 days of storage, the TVBN content of USNS samples and SNS samples was 58.16 mg N/100 g and 40.42 mg N/100 g flesh, respectively, while the

Table 4
Total volatile basic nitrogen[A](TVBN) (mg N/100 g) of chub mackerel stored at 2 ± 0.5 °C as a function of storage time

Storage time[B] (days)	Salted, smoked (method 1)	Salted, smoked (method 2)	Salted, non-smoked	Unsalted, non-smoked
0	10.93 ± 0.36[a]	10.93 ± 0.36[a]	10.93 ± 0.36[a]	10.93 ± 0.36[a]
1	20.94 ± 0.50[a]	20.18 ± 0.32[a]	11.28 ± 0.24[b]	11.15 ± 0.40[b]
6	19.78 ± 0.43[a]	20.26 ± 0.51[a]	12.72 ± 0.29[b]	15.93 ± 0.57[c]
12	21.52 ± 0.36[a]	21.81 ± 0.32[a]	14.26 ± 0.36[b]	24.20 ± 0.29[c]
18	19.64 ± 0.51[a]	19.33 ± 0.76[a]	21.59 ± 0.64[b]	36.18 ± 0.70[c]
24	21.26 ± 0.64[a]	21.40 ± 0.42[a]	31.60 ± 0.67[b]	45.92 ± 0.65[c]
30	20.88 ± 0.48[a]	21.95 ± 0.60[a]	40.42 ± 0.38[b]	58.16 ± 0.71[c]

[a–c] Values in the same line followed by different letter are significantly different ($p < 0.05$).
[A] Values represent the mean of six determinations ($n = 2 \times 3$) ± SD.
[B] Values on day 0 correspond to unsalted, non-smoked product.

corresponding values of the two smoked samples were 20.88 and 21.95 mg N/100 g.

The TVBN content of smoked samples (for both smoking methods) remained significantly lower than the acceptability limit of 35 mg N/100 g of muscle set by the EU (EEC, 1995). This is in agreement with the sensory scores. The corresponding TVBN levels of non-smoked samples exceeded the above limit of 35 mg N/100 g after approximately 18 days of storage (USNS samples, 36.18 mg N/100 g), while the SNS samples were approached this limit between 26 and 27 days of storage.

Various authors have reported different acceptability levels for TVBN value: 35–40 mg N/100 g (Connell, 1990); 25–30 mg N/100 g (Lopez-Caballero, Perez-Mateos, Montero, & Borderias, 2000); 20–25 mg N/100 g (Kim, Paik, & Lee, 2002). Such differences reflect different products, specific treatments and processing conditions.

3.5. Determination of trimethylamine nitrogen

Trimethylamine nitrogen (TMAN) values are presented in Table 5. As results show the initial TMA content of chub mackerel fillets was low (1.22 mg N/100 g of sample) indicative of the freshness of the samples. According to Connell (1990) a value of 1.5 mg TMA nitrogen/100 g of product has been recommended as an upper limit for very good quality cod. Of course it has been shown that levels of TMAN depend on species, age, time of year, muscle type and diet of fish (Reddy et al., 1997; Rodriguez, Besteiro, & Pascual, 1999). TMAN is produced from trimethylamine oxide (TMAO) possibly partly by the action of intrinsic enzymes but certainly through bacterial action (Connell, 1990; Rodriguez et al., 1999).

While TMAN levels of smoked samples (both methods) remained practically constant with storage time, the corresponding TMAN levels of non-smoked samples increased progressively during storage. This increase was greater in the USNS samples which exceeded the legal limit of 12 mg N per 100 g, set by the EU for fish (EEC, 1995) after approximately 19–20 days of storage. In contrast, the SNS samples reached this limit after 30 days of storage.

In general, the TMAN content of USNS samples, was significantly higher ($p < 0.05$) than TMAN content of SNS samples. This observation is in complete agreement with results reported by Leroi and Joffraud (2000a) and Hansen et al. (1995) for smoked salmon and is indicative of the preservative effect of salt. According to Leroi et al. (2000b), the total viable counts (TVC) of smoked salmon was lowered by 3.3 log with the addition of 5% NaCl (g per 100 g of finished product). This salt content resembles that obtained in the present study (5.7 and 5.8 g per 100 g of finished product).

After 30 days of storage the highest TMAN value was recorded in the USNS samples (17.11 mg N/100 g) while the lowest TMAN value was recorded in the smoked samples 3.28 and 3.64 mg N/100 g for smoking method 1 and 2, respectively. These TMAN results correlate well with those reported by Hansen et al. (1995).

There were no statistically significant differences ($p < 0.05$) in the TMAN values between the two smoking methods.

3.6. Determination of 2-thiobarbituric acid

2-Thiobarbituric acid values for chub mackerel are presented in Table 6. No statistically significant differences ($p < 0.05$) in TBA values were observed between the two smoking methods. In contrast, TBA values of non-smoked (salted or unsalted) samples were significantly ($p < 0.05$) higher than corresponding values of smoked samples. This fact can be attributed to the antioxidant activity of phenolic constituents of smoke deposited onto the fish during the smoking process.

As can be seen in Table 6, there is a trend towards an increase in TBA values up to a certain point during the storage period; followed by either, a decrease in these values or a lower increase rate. Given that TBA index is a measure of malondialdehyde (MDA) which is an end-product of lipid oxidation (Fernandez, Perez-Alvarez,

Table 5
Trimethylamine nitrogen[A] (TMAN) (mg N/100 g) of chub mackerel stored at 2 ± 0.5 °C as a function of storage time

Storage time[B](days)	Salted, smoked (method 1)	Salted, smoked (method 2)	Salted, non-smoked	Unsalted, non-smoked
0	1.22 ± 0.06[a]	1.22 ± 0.06[a]	1.22 ± 0.06[a]	1.22 ± 0.06[a]
1	2.96 ± 0.08[a]	3.15 ± 0.16[a]	1.56 ± 0.09[b]	1.48 ± 0.05[b]
6	3.19 ± 0.15[a]	3.39 ± 0.11[a]	1.90 ± 0.22[b]	2.95 ± 0.06[c]
12	2.65 ± 0.12[a]	2.92 ± 0.23[a]	3.36 ± 0.20[b]	6.87 ± 0.22[c]
18	3.58 ± 0.24[a]	3.26 ± 0.17[a]	5.78 ± 0.28[b]	11.35 ± 0.40[c]
24	3.40 ± 0.16[a]	3.52 ± 0.13[a]	9.80 ± 0.38[b]	13.82 ± 0.33[c]
30	3.28 ± 0.12[a]	3.64 ± 0.24[a]	11.92 ± 0.49[b]	17.11 ± 0.30[c]

[a–c] Values in the same line followed by different letter are significantly different ($p < 0.05$).
[A] Values represent the mean of six determinations ($n = 2 \times 3$) ± SD.
[B] Values on day 0 correspond to unsalted, non-smoked product.

Table 6
2-Thiobarbituric acid (TBA)[A] (mg of malondialdehyde/kg) of chub mackerel stored at 2 ± 0.5 °C as a function of storage time

Storage time[B](days)	Salted, smoked (method 1)	Salted, smoked (method 2)	Salted, non-smoked	Unsalted, non-smoked
0	0.23 ± 0.05[a]	0.23 ± 0.05[a]	0.23 ± 0.05[a]	0.23 ± 0.05[a]
1	0.54 ± 0.05[a]	0.47 ± 0.05[a]	0.30 ± 0.09[b]	0.26 ± 0.04[b]
6	0.49 ± 0.18[a]	0.56 ± 0.07[a]	0.52 ± 0.15[a]	0.50 ± 0.11[a]
12	0.60 ± 0.08[a]	0.51 ± 0.15[a]	1.03 ± 0.11[b]	0.71 ± 0.07[a]
18	0.57 ± 0.12[a]	0.65 ± 0.05[a]	1.31 ± 0.12[b]	0.93 ± 0.16[c]
24	0.70 ± 0.07[a]	0.61 ± 0.14[a]	1.19 ± 0.07[b]	1.11 ± 0.10[b]
30	0.75 ± 0.15[a]	0.83 ± 0.09[a]	1.44 ± 0.20[b]	1.03 ± 0.08[c]

[a–c] Values in the same line followed by different letter are significantly different ($p < 0.05$).
[A] Values represent the mean of six determinations ($n = 2 \times 3$) ± SD.
[B] Values on day 0 correspond to unsalted, non-smoked product.

& Fernandez-Lopez, 1997), decrease in MDA content may be caused by interaction between MDA and proteins, aminoacids, glycogen, etc. (Fernandez et al., 1997; Gomes, Silva, Nascimento, & Fukuma, 2003). This observation is in agreement with results reported by other authors (Curzio & Quaranta, 1982; Fernandez et al., 1997; Gomes et al., 2003).

The initial TBA value of raw chub mackerel was 0.23 mg MDA/kg. This value increased to 0.54 and 0.47 mg MDA/kg after the smoking process (method 1 and 2, respectively). The increase in TBA value during the smoking procedure may be attributed to the partial dehydration of fish and to the increased oxidation of unsaturated fatty acids as a result of smoking at relatively high temperatures (up to 70 °C). This observation is in agreement with results reported by Goktepe and Moody (1998) who observed a two fold increase in TBA value of raw catfish after smoking (smoke temperature up to 82 °C).

A statistically significant ($p < 0.05$) but moderate increase was observed in TBA values of smoked samples during storage. Final TBA values of 0.75 and 0.83 mg MDA/kg for smoked fish did not exceed the value of 1–2 mg MDA/kg which is usually regarded as the limit beyond which the fish will normally develop an objectionable odour/taste (Connell, 1990). In contrast, the TBA values of non-smoked samples reached 1 mg MDA/kg after approximately 21 days (USNS samples) and after 12 days (SNS samples). The above reported observations may be attributed to the phenolic constituents deposition on the smoked fish. Among the smoked components the phenols have the highest antioxidant activity (Doe, 1998).

It is interesting to note that in contrast to other parameters examined (TVBN, TMAN, etc.) the TBA values of SNS samples were significantly ($p < 0.05$) higher than those of USNS samples after 12 days of storage. This fact can be attributed to the presence of salt in the SNS samples and is in agreement with results reported by other authors (Aubourg & Ugliano, 2002; Connell, 1990; Davis, Goodwin, Smith, & Hole, 1993) who observed that salt contact with fish flesh enhances lipid oxidation.

Another interesting observation is that vacuum packaged non-smoked fish samples produced significant quantities of 2-thiobarbituric acid-reactive substances. This observation suggests that in chilled fish products rancidity does not depend only on the amount of oxygen in the package but also on the type of microbial flora present (Ruiz-Capillas & Moral, 2001a).

3.7. Sensory evaluation

The results of the sensory evaluation of samples are given in Table 7. As the results show the smoked samples (both smoking methods) were scored as excellent or very good throughout the entire storage period. The mean score for taste of smoked samples was 8.0 and 9.5 for smoking methods 1 and 2, respectively, after 30 days of storage. The corresponding values for odour of smoked samples were 8.1 and 9.9; 8.4 and 7.0 for texture and 7.8 and 9.8 for flesh colour. Similar sensory characteristics of the two methods of smoking may be attributed to the adequate cooking provided by the smoking process used.

In general as time of storage progressed, the sensory properties of samples smoked with method 2 received a higher score ($p < 0.05$) than samples smoked with method 1 with the exception of texture. This may be attributed to the fish/smoke higher contacting time and temperature in method 2. It is well known that higher temperatures increase the deposition of smoke compounds (Cardinal et al., 2001). This results in a more distinct smoking odour, taste and golden brown color. As results show these characteristics remained practically unchanged during the entire storage period for samples smoked with method 2. On the other hand, the texture of samples smoked with method 2, received a lower ($p < 0.05$) score after 18 days of storage due to a toughening effect of smoke on the muscle proteins. Some authors have attributed this phenomenon to formaldehyde, which acts as a protein denaturant by virtue of its reaction with amino groups (Horner, 1997; Robertson, 1993). Results show that the deposition of formaldehyde was higher in smoking method 2.

Table 7
Sensory evaluation[A] of vacuum packaged chub mackerel stored at 2 ± 0.5 °C as a function of storage time

Sensory parameter	Preservation method	Storage time (days)					
		0	6	12	18	24	30
Taste	Smoking method 1	10.0 ± 0.0[a]	10.0 ± 0.0[a]	10.0 ± 0.0[a]	9.8 ± 0.2[a]	9.1 ± 0.3[b]	8.0 ± 0.6[c]
	Smoking method 2	10.0 ± (0.0)[a]	10.0 ± 0.0[a]	9.9 ± 0.1[a]	9.5 ± 0.4[a]	9.7 ± 0.3[a]	9.5 ± 0.4[a]
	SNS samples	–	–	–	–	–	–
	USNS samples	–	–	–	–	–	–
Odour	Smoking method 1	10.0 ± (0.0)[a]	10.0 ± 0.0[a]	10.0 ± 0.0[a]	9.5 ± 0.2[a]	8.6 ± 0.4[b]	8.1 ± 0.6[b]
	Smoking method 2	10.0 ± 0.0[a]	10.0 ± 0.0[a]	10.0 ± 0.0[a]	10.0 ± 0.0[a]	9.9 ± 0.1[a]	9.9 ± 0.1[a]
	SNS samples	10.0 ± 0.0[a]	9.0 ± 0.3[b]	7.8 ± 0.4[c]	7.3 ± 0.7[c]	5.5 ± 0.6[d]	3.4 ± 0.4[e]
	USNS samples	10.0 ± 0.0[a]	8.2 ± 0.5[b]	7.1 ± 0.3[c]	5.4 ± 0.9[d]	3.3 ± 0.5[e]	2.2 ± 0.3[f]
Flesh texture	Smoking method 1	10.0 ± 0.0[a]	10.0 ± 0.0[a]	9.8 ± 0.2[a]	9.9 ± 0.1[a]	9.4 ± 0.4[a]	8.4 ± 0.2[b]
	Smoking method 2	10.0 ± 0.0[a]	10.0 ± 0.0[a]	9.4 ± 0.2[a]	8.5 ± 0.5[b]	8.1 ± 0.3[b]	7.0 ± 0.6[c]
	SNS samples	10.0 ± 0.0[a]	10.0 ± 0.0[a]	8.5 ± 0.5[b]	7.4 ± 0.3[c]	5.6 ± 0.7[d]	1.5 ± 0.3[e]
	USNS samples	10.0 ± 0.0[a]	9.1 ± 0.9[a]	7.0 ± 0.6[b]	5.9 ± 0.4[c]	3.3 ± 0.8[d]	2.5 ± 0.4[d]
Flesh colour	Smoking method 1	10.0 ± 0.0[a]	10.0 ± 0.0[a]	10.0 ± 0.0[a]	9.5 ± 0.3[a]	9.3 ± 0.4[a]	7.8 ± 0.6[b]
	Smoking method 2	10.0 ± 0.0[a]	10.0 ± 0.0[a]	10.0 ± 0.0[a]	9.8 ± 0.2[a]	9.9 ± 0.1[a]	9.8 ± 0.2[a]
	SNS samples	10.0 ± 0.0[a]	10.0 ± 0.0[a]	8.0 ± 0.8[b]	8.0 ± 0.6[b]	6.4 ± 0.8[c]	5.0 ± 0.3[d]
	USNS samples	10.0 ± 0.0[a]	8.5 ± 0.3[b]	7.5 ± 0.5[c]	7.0 ± 0.3[c]	6.0 ± 0.4[d]	4.1 ± 0.7[e]

[a–f] Values in the same line followed by different letter are significantly different ($p < 0.05$).
[A] Scale from 10 to 0 (10 excellent and 0 very bad). Rejection point 6.

As the results in Tables 4–7 show, there is a good correlation between sensory scores and chemical indices of quality determined. Odour and texture proved to be the most sensitive of the sensory properties evaluated. Based on present data the shelf life of USNS fillets was 15–16 days while the shelf life of SNS fillets was 21–22 days. The upper limit of 30–35 mg for TVBN was reached after approximately 18 and 24 days, respectively, for the USNS and SNS sample. The upper limit of 12 mg for TMA was reached after approximately 18–20 and 30 days, respectively, for the USNS and SNS samples.

4. Conclusions

Present results indicate that application of smoking method 2 resulted in a product with more distinct smoked odour, taste and colour which were retained throughout the 30 day storage period of the study. The prolongation of smoking time (method 2) resulted in the deposition of more smoke constituents with antioxidant and antimicrobial activity (phenols, formaldehyde, etc.) which contributed to the extension of shelf-life of the product. Both smoking methods produced good quality chub mackerel products for the entire storage period.

The only disadvantage of the smoking method 2 was the slightly more tough texture, which however remained within acceptable limits throughout the entire storage period. In general smoking method 2 produced products with better sensory characteristics while no significant differences were observed in the biochemical indices between two smoking methods. With regard to the non-smoked products, based on both sensory assessment and chemical indices determination, the shelf life of USNS chub mackerel fillets is approximately 15–16 days and of SNS fillets is 21–22 days.

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References

Anonymous (2000). *Preservation and trade of seafoods*. Athens: Greek Ministry of Agriculture, Department of Fishing.

AOAC (1995). *Official methods of analysis*. Gaithersburg, MD: Association of Official Analytical Chemists. Official methods 937.09 and 985.14.

Aubourg, S. P., & Ugliano, M. (2002). Effect of brine pre-treatment on lipid stability of frozen horse mackerel (*Trachurus trachurus*). *European Food Research and Technology, 215*, 91–95.

Cardinal, M., Knockaert, C., Torrissen, O., Sigurgisladottir, S., Morkore, T., Thomassen, M., & Vallet, J. L. (2001). Relation of smoking parameters to the yield colour and sensory quality of smoked Atlantic salmon (*Salmo salar*). *Food Research International, 34*, 537–550.

Connell, J. J. (1990). Methods of assessing and selecting for quality. In *Control of fish quality* (3rd ed., pp. 122–150). Oxford: Fishing News Books.

Curzio, O. A., & Quaranta, H. O. (1982). Delay of oxidative rancidity in irradiated hake (Merluccias merluccias hubbsi). *Lebensmittel-Wissenschaft und-Technologie, 15*, 171–172.

Davis, L., Goodwin, L., Smith, G., & Hole, M. (1993). Lipid oxidation in salted-dried fish; the effect of temperature and light on the rate of

oxidation of a fish oil. *Journal of the Science of Food and Agriculture, 62*, 355–359.

Doe, P. E. (Ed.). (1998). *Fish drying and smoking, production and quality* (pp. 13–115). Lancaster, PA: Technomic Publishing Co, Inc., pp. 89–115.

Duffes, F. (1999). Improving the control of Listeria monocytogenes in cold smoked salmon. *Trends in Food Science and Technology, 10*, 211–216.

EEC (1995). Total volatile basic nitrogen (TVB-N) limit values for certain categories of fishery products and specifying the analysis methods to be used. Commission Decision 95/149/EEC of 8 March 1995, *Official Journal of European Communities, L97*, 84–87.

Espe, M., Nortvedt, R., Lie, O., & Hafsteinsson, H. (2002). Atlantic salmon (*Salmo salar*, L) as raw material for the smoking industry. II: effect of different smoking methods on losses of nutrients and on the oxidation of lipids. *Food Chemistry, 77*, 41–46.

Fernandez, J., Perez-Alvarez, J. A., & Fernandez-Lopez, J. A. (1997). Thiobarbituric acid test for monitoring lipid oxidation in meat. *Food Chemistry, 59*(3), 345–353.

Goktepe, I., & Moody, M. W. (1998). Effect of modified atmosphere packaging on the quality of smoked catfish. *Journal of Muscle Foods, 9*, 375–389.

Gomes, H. A., Silva, E. N., Nascimento, M. R. L., & Fukuma, H. T. (2003). Evaluation of the 2-thiobarbituric acid method for the measurement of lipid oxidation in mechanically deboned gamma irradiated chicken meat. *Food Chemistry, 80*, 433–437.

Guillen, M. D., & Errecalde, M. C. (2002). Volatile components of raw and smoked black bream (*Brama raii*) and rainbow trout (*Oncorhynchus mykiss*) studied by means of solid phase microextraction and gas chromatography/mass spectrometry. *Journal of the Science of Food and Agriculture, 82*, 945–952.

Hansen, L. T., Gill, T., & Huss, H. H. (1995). Effects of salt and storage temperature on chemical, microbiological and sensory changes in cold-smoked salmon. *Food Research International, 28*(2), 123–130.

Hattula, T., Elfving, K., Mroueh, U.-M., & Luoma, T. (2001). Use of liquid smoke flavouring as an alternative to traditional flue gas smoking of Rainbow Trout fillets (*Oncorhynchus mykiss*). *Lebensmittel-Wissenschaft und-Technologie, 34*, 521–525.

Horner, W. F. A. (1997). Preservation of fish by curing, drying, salting and smoking. In G. M. Hall (Ed.), *Fish processing technology* (2nd ed., pp. 32–73). London: Blackie Academic and Professional.

Hyytia, E., Hielm, S., Mokkila, M., Kinnunen, A., & Korkeala, H. (1999). Predicted and observed growth and toxigenesis by Clostridium botulinum type E in vacuum-packaged fishery products challenge tests. *International Journal of Food Microbiology, 47*, 161–169.

Kim, Y.-M., Paik, H.-D., & Lee, D.-S. (2002). Shelf-life characteristics of fresh oysters and ground beef as affected by bacteriocin-coated plastic packaging film. *Journal of the Science of Food and Agriculture, 82*, 998–1002.

Kolodziejska, I., Niecikowska, C., Januszewska, E., & Sikorski, Z. E. (2002). The microbial and sensory quality of Mackerel hot smoked in mild conditions. *Lebensmittel-Wissenschaft und-Technologie, 35*, 87–92.

Leroi, F., & Joffraud, J. J. (2000a). Salt and smoke simultaneously affect chemical and sensory quality of cold-smoked salmon during 5 °C storage predicted using factorial design. *Journal of Food Protection, 63*(9), 1222–1227.

Leroi, F., Joffraud, J. J., & Chevalier, F. (2000b). Effect of salt and smoke on the microbiological quality of cold-smoked salmon during storage at 5 °C as estimated by the factorial design method. *Journal of Food Protection, 63*(4), 502–508.

Lopez-Caballero, M. E., Perez-Mateos, M., Montero, P., & Borderias, A. J. (2000). Oyster preservation by high-pressure treatment. *Journal of Food Protection, 63*(2), 196–201.

Malle, P., & Tao, S. H. (1987). Rapid quantitative determination of trimethylamine using steam distillation. *Journal of Food Protection, 50*(9), 756–760.

Metin, S., Erkan, N., Varlik, C., & Aran, N. (2001). Extension of shelf-life of chub mackerel (Scomber japonicus Houttuyn 1780) treated with lactic acid. *European Food Research and Technology, 213*, 174–177.

Plahar, W. A., Nerquaye-Tetteh, G. A., & Annan, N. T. (1999). Development of an integrated quality assurance system for the traditional *Sardinella* sp. and anchovy fish smoking industry in Ghana. *Food Control, 10*, 15–25.

Reddy, N. R., Roman, M. G., Villanueva, M., Solomon, H. M., Kautter, D. A., & Rhodehamel, E. J. (1997). Shelf life and clostridium botulinum toxin development during storage of modified atmosphere-packed fresh catfish fillets. *Journal of Food Science, 62*(4), 878–884.

Robertson, G. L. (1993). Packaging of flesh foods. In *Food packaging, principles and practice* (pp. 463–466). New York: Marcel Dekker Inc..

Rodriguez, C. J., Besteiro, I., & Pascual, C. (1999). Biochemical changes in freshwater rainbow trout (*Oncorhynchus mykiss*) during chilled storage. *Journal of the Science of Food and Agriculture, 79*, 1473–1480.

Rorvik, L. M. (2000). Listeria monocytogenes in the smoked salmon industry. *International Journal of Food Microbiology, 62*, 183–190.

Ruiz-Capillas, C., & Moral, A. (2001a). Residual effect of CO_2 on hake (*Merluccius merluccius* L.) stored in modified and controlled atmospheres. *European Food Research and Technology, 212*, 413–420.

Ruiz-Capillas, C., Morales, J., & Moral, A. (2001b). Combination of bulk storage in controlled and modified atmospheres with modified atmosphere packaging system for chilled whole gutted hake. *Journal of the Science of Food and Agriculture, 81*, 551–558.

Sigurgisladottir, S., Sigurdardottir, M. S., Torrissen, O., Vallet, J. L., & Hafsteinsson, H. (2000). Effects of different salting and smoking processes on the microstructure, the texture and yield of Atlantic salmon (*Salmo salar*) fillets. *Food Research International, 33*, 847–855.