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Combined effect of light salting, modified atmosphere packaging and oregano essential oil on the shelf-life of sea bream (*Sparus aurata*): Biochemical and sensory attributes

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Abstract

The combined effect of modified atmosphere packaging (MAP: 40% CO_2/30% O_2/30% N_2) and oregano essential oil, on the shelf-life of lightly salted cultured sea bream (*Sparus aurata*) fillets stored under refrigeration was studied. Quality assessment was based on sensory analysis and biochemical indices determination. Total volatile basic nitrogen (TVBN) and trimethylamine nitrogen (TMAN) values were higher in sea bream fillets stored in air followed by salted fillets stored in air. For salted sea bream fillets stored under MAP the inhibition in the TVBN and TMAN values was evident in the order MAP < MAP/0.4% (v/w) oregano oil < MAP/0.8% (v/w) oregano oil indicating the preservative effect of oregano oil. Salting had a noticeable preservative effect but produced an increase in the 2-thiobarbituric acid (TBA) values while oregano oil had a strong antioxidant activity giving the lowest TBA values. All raw sea bream fillet samples received acceptable sensory scores during the first 15–16 days of storage. The salted samples remained acceptable up to ca. 20–21 days while the MAP salted samples up to ca. 27–28 days of storage. The oregano oil addition in MAP salted samples yielded a distinct but pleasant flavor and contributed to a considerable slower process of fish spoilage given that the fillets treated with 0.8% (v/w) oregano oil were still sensory acceptable after 33 days of storage. The preservative effect was greater as the oregano oil concentration was greater.

Keywords: Modified atmosphere packaging; Oregano essential oil; Salting; Shelf-life; Sea bream; Biochemical analysis; Sensory assessment

1. Introduction

Sea bream (*Sparus aurata*) is one of the major farmed fish in the Mediterranean countries. The aquaculture production of sea bream in Greece has rapidly increased during the last decade from 6700 tons in 1994 to 33,000 tons in 2000 corresponding to the highest production among the Mediterranean countries (Anonymous, 2001).

Given that the shelf-life of refrigerated sea bream is relatively short and that there is a growing tendency of consumers for consumption of fresh rather than processed and frozen fish, research on the application of new preservation methods, which permit shelf-life extension of fresh fish, is required.

Modified atmosphere packaging (MAP) is a widely used food preservation method, which in combination with refrigeration could provide a substantial shelf-life extension of fish. Thus numerous studies have been conducted on the effect of MAP on fish and fish products (Boknaes et al., 2002; Debevere & Boskou, 1996; Goulas, Chouliara, Nessi, Kontominas, & Savvaidis, 2005; Masniyom, Benjakul, & Visessanguan, 2002; Mejlholm & Dalgaard, 2002; Ozogul, Polat, & Ozogul, 2004; Pastoriza, Sampedro, Herrera, & Cabo, 1998; Ruiz-Capillas & Moral, 2001, 2005; Sivertsvik, Jeksrud, & Rosnes, 2002; Tassou, Drosinos, & Nychas, 1996).

Fish are very susceptible to both microbiological and chemical deterioration, due to their chemical composition.

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The specific shelf-life extension of refrigerated fish products achieved through the use of MAP depends on raw material (species, fat content, initial microbiological populations, etc.), temperature, gas mixture and packaging materials used (Davies, 1997; Sivertsvik et al., 2002). In order to increase shelf-life of fresh fish provided by MAP, low levels of salt and/or natural, mostly, preservatives (antimicrobial and antioxidants) have been used. Thus, oregano, thyme, garlic, bay leaf, rosemary, marjoram, clove, etc., or their extracts, known as essential oils (EO), have been used alone or in combination with other preservation methods such as MAP, salting, irradiation, etc., to improve the sensory characteristics and extend the shelf-life of foods (Burt, 2004; Gimenez, Roncales, & Beltran, 2004; Mahmoud et al., 2004; Mejlholm & Dalgaard, 2002; Miguel et al., 2004; Vareltzis, Koufidis, Gavriilidou, Papavergou, & Vasiliadou, 1997; Wong & Kitts, 2002).

Among the EO from various aromatic plants, oregano oil a characteristic spice of the Mediterranean cuisine is widely used in raw or cooked foods yielding a distinct but pleasant aroma and taste. Oregano EO has been studied for its antimicrobial and antioxidant activity in various commercial or model foods, e.g., raw and cooked chicken, beef, fish, fish oil, sunflower oil, egg yolk, etc. (Burt, 2004; Harpaz, Glatman, Drabkin, & Gelman, 2003; Kulisic, Radonic, Katalinic, & Milos, 2004; Mejlholm & Dalgaard, 2002; Skerget et al., 2005; Tassou et al., 1996; Tsimidou, Papavergou, & Boskou, 1995; Wong & Kitts, 2002).

The four major constituents of oregano EO as percentage of total content are the phenols carvacrol and thymol and the monoterpene hydrocarbons *p*-cymene and γ-terpinene (Baydar, Sagdic, Ozkan, & Karadogan, 2004; Burt, 2004; Kokkini, Karousou, Dardioti, Krigas, & Lanaras, 1997). Carvacrol and thymol comprise the main antimicrobial and antioxidant components while possible synergistic antibacterial action has been attributed to terpenes (Burt, 2004; Mahmoud et al., 2004; Skerget et al., 2005; Wong & Kitts, 2002). Also, the flavonoids of oregano EO are a group of compounds with antioxidant activity (Skerget et al., 2005; Vekiari, Oreopoulou, Tzia, & Thomopoulos, 1993).

Despite the numerous studies in the literature on the antibacterial and antioxidant activity of oregano EO and subsequent effect on the shelf-life of foods, very little data exists on the effect of oregano EO on the shelf-life of fish and fish products (Harpaz et al., 2003; Mahmoud et al., 2004; Tsimidou et al., 1995) and on the effect of oregano EO on the shelf-life of MAP refrigerated fish (Mejlholm & Dalgaard, 2002; Tassou et al., 1996).

Thus, the objective of the present work was (1) to study the combined effect of light salting, MAP and oregano EO on the shelf-life and quality retention of sea bream fillets at 4 °C by sensory analysis of raw fillet samples and biochemical indices determination and (2) to correlate sensory to biochemical indices data.

2. Materials and methods

2.1. Fish samples

Fresh sea bream was obtained from the aquaculture farm BURSINOS S.A. in Igoumenitsa, Greece in July 2004. Fish were killed by immersion in ice-cold water (hypothermia) and transported to the laboratory within 1.5 h in foamed polystyrene boxes containing ice. The average weight of the whole fish was 305 g. Average length was 250 mm.

2.2. Preparation of fish samples and packaging

The fish were headed and filleted manually using a sterile scalp. The average weight of filleted fish was 59.6% of the initial average weight. The fillets were separated into five lots.

Salting of the four filleted lots was carried out by immersion in a brine containing 100 g/L NaCl at 8 °C for 1 h with a fish:brine ratio of 1:1 (w/v). All filleted samples including the control were packaged in Low density Polyethylene/Polyamide/Low density Polyethylene (LDPE/PA/LDPE) barrier pouches 25 cm × 35 cm (two fillets per pouch, weighing ca. 180 g) 75 μm in thickness having an oxygen permeability of 52.2 $cm^3\ m^{-2}\ day^{-1}\ atm^{-1}$ at 75% relative humidity (RH), 23 °C, a carbon dioxide permeability of 191 $cm^3\ m^{-2}\ day^{-1}\ atm^{-1}$ at 0% relative humidity (RH), 23 °C and a water vapour permeability of 2.4 $g\ m^{-2}\ day^{-1}$ at 100% RH, 23 °C.

Oregano oil was added to the two lots of filleted samples in appropriate volumes to the surface (two sides) of each fillet using a pipette so as to achieve a 0.4% and 0.8% oil volume per fish flesh weight (v/w) respectively. Samples were as follows: A1 (control samples: air packaged), A2 (salted, air packaged), M1 [salted, packaged in 40% CO_2/30% O_2/30% N_2 (MAP)], M2 (salted, packaged in MAP after addition of 0.4% oregano oil) and M3 (salted, packaged in MAP after addition of 0.8% oregano oil).

The above reported gas mixture was selected given the proximate analysis of sea bream (Table 1, relatively low fat content) and literature data (Tassou et al., 1996).

Pouches containing fish fillets for MAP samples were first evacuated and immediately injected the gas mixture which was produced using a PBI Dansensor MAP Mix 9000 Gas mixer (Dansensor, Denmark) connected to 3 tanks containing CO_2, O_2 and N_2, respectively. The ratio between the volume of gas mixture to product was 2:1.

Table 1
Proximate analysis[a] (%) of muscle of cultured sea bream

Composition	Mean value (%)
Protein	19.9 ± 1.3
Fat	6.2 ± 0.8
Moisture	72.4 ± 0.7
Ash	1.4 ± 0.1

[a] Values represent the mean of six determinations ± SD

Pouches were then heat-sealed using a BOSS model NE 48 vacuum sealer (BOSS, Germany) and kept under refrigeration (4 ± 0.5 °C) for a period of 33 days.

Sampling was carried out at predetermined time intervals namely: 0, 4, 8, 12, 16, 20, 24, 28 and 33 days. At each sampling day 3 fish fillets were randomly chosen for analysis. The experiment was duplicated for each fillet on different occasions ($3 \times 2 = 6$ samples).

2.3. Sodium chloride content and drip loss determination

Sodium chloride content was determined using the method of AOAC (1995).

Drip loss of fish fillets was determined as follows: liquid was collected from the pouches after draining at the time of sampling and the weight of the liquid was expressed as % of initial fillet weight (Dalgaard, Gram, & Huss, 1993).

2.4. pH determination

pH was determined using the method of AOAC (1995) after appropriate modification (Goulas & Kontominas, 2005).

2.5. Proximate analysis

Moisture content was determined by oven drying of 5 g of minced fish fillet at 105 °C for 20–24 h until constant weight.

Total crude protein was determined using the method of AOAC (1995).

The ash content was determined by ashing of 5 g of minced fish in a furnace at 550 °C for 24 h using magnesium acetate as an ashing aid.

The total lipids were extracted from a 20 g sample of the minced fillets using chloroform: methanol (2:1 by vol) extraction solution (Vareltzis et al., 1997).

2.6. Biochemical analysis

2.6.1. Determination of total volatile basic nitrogen

Total volatile basic nitrogen (TVBN) was determined by distillation after the addition of MgO to minced fish sample. The distillate was collected in a flask containing a 3% aqueous solution of boric acid and a mixed indicator produced from dissolution of 0.1 g of methyl red and 0.05 g of methylene blue to 100 ml of ethanol. Finally, the boric acid solution was titrated with a 0.1 N hydrochloric acid solution. Analysis was carried out according to the method described by Pearson (1976) after appropriate modification (Goulas & Kontominas, 2005).

2.6.2. Determination of trimethylamine nitrogen

Trimethylamine nitrogen (TMAN) was determined using the above mentioned method after appropriate modification: formaldehyde was added to block the primary and secondary amines. Analysis was carried out according to the method described by Malle and Tao (1987) after appropriate modification (Goulas & Kontominas, 2005).

2.6.3. Determination of 2-thiobarbituric acid

The method of Gomes, Silva, Nascimento, and Fukuma (2003) was used after appropriate modification. The method is based on the spectrophotometric quantitation of the pink complex formed after reaction of one molecule of malondialdehyde (MDA), product of distillation, with two molecules of 2-thiobarbituric acid (TBA) added to the distillate.

2.6.3.1. Preparation of TBA solution. The TBA solution was prepared by weighing 0.3 g of TBA (Merck, Germany) 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 so as to achieve a 0.021 M TBA solution.

2.6.3.2. Determination of TBA. Fish flesh (50 g) was blended after the addition of 6 ml of ethanolic solution of butylated hydroxytoluene (BHT, 1 g l^{-1}) to prevent autoxidation. Aliquots of homogenized fish flesh (10 g) were transferred to a flat-bottomed flask and one drop of silicone anti-foaming agent (Merck, Germany) added plus 2.5 ml HCl 4 N and 97.5 ml distilled water. This sample was then distilled and the first 50 ml of distillate collected. Distillation was carried out in triplicate. Then 5 ml of the distillate plus 0.6 ml BHT (1 g l^{-1}) were added to 5 ml of 0.021 M TBA solution into a screw-cap test tube and heated in a water bath (90 °C) for 40 min for pink colour development. The test tube was then cooled and the optical density was determined at 532 nm on a SECOMAM ANTHELIE Spectrophotometer model 70ST0375 (Secomam, France) using as control a solution containing 5 ml distilled water, 5 ml TBA solution and 0.6 ml BHT (1 g l^{-1}). TBA values were expressed as mg of malondialdehyde (MDA)/kg of sample. The concentration of MDA was calculated from a standard curve using 1,1,3,3-tetraethoxy-propane (TEP) as the standard compound.

2.7. Sensory evaluation

The sensory quality of raw sea bream fillets was evaluated at each sampling time (day 0, 4, 8, 12, 16, 20, 24, 28, 33) by a seven member trained panel. Panellists were trained for a period of 3 months in 1-h sessions three times a week (36 h total). Triangle tests were performed in order to select 7 panellists who could detect off-flavors in raw fish. Prior to sample evaluation, the 7 selected panellists participated in orientation sessions to familiarize themselves with the flavour (off-odour), texture and colour attributes of raw fish. Fillet samples (140 g) taken from the flesh of the anterior dorsal region of each fillet were presented individually to the panellists (each panellist evaluating

approximately 20 g of fillet sample) in plastic cups covered with a lid in random order. Freshly blast frozen (−30 °C) sea bream fillet samples were also presented to the panellists after thawing at room temperature, this serving as the control sample. Sensory evaluation was conducted in individual booths under controlled conditions of light, temperature and humidity.

Panellists were asked to score odour, flesh colour and texture of fillets using a 0–10 intensity scale with 10 corresponding to the most liked sample and 0 corresponding to least liked sample. The product was defined as unacceptable (a score of <6) after development of first off-odour or under application of finger pressure, muscle returns more than half way (muscle texture) or slime production or after first discoloration (muscle colour).

2.8. Statistical analysis

Experiments were replicated twice on different occasions. All analyses were run in triplicate for each replicate ($n = 2 \times 3$). Data excluding sensory were subjected to analysis of variance (ANOVA) using the software Statgraphics (Statistical Graphics Corp. Rockville, MD, USA). Means and standard deviations were calculated for all data, and, when F-values were significant at the $P < 0.05$ level, mean differences between pairs were separated by the least significant difference (LSD) procedure.

3. Results and discussion

3.1. Proximate analysis

Proximate analysis results (day 0) are presented in Table 1. The results are in good agreement with those reported by other authors (Kyrana, Lougovois, & Valsamis, 1997; Tejada & Huidobro, 2002). The fat content of aquacultured sea bream is higher as compared with fat content of wild sea bream and is attributed to the specific nutrition of farmed fish.

3.2. Determination of pH

The initial pH of untreated fillets on day 0 was 6.12 indicating the freshness of fish samples (results not shown). During storage at 4 °C the pH of control and treated samples increased to reach values from 6.28 for treated with 0.8% v/w oregano oil MAP samples to 6.88 for control samples after 33 days of storage. The higher ($p < 0.05$) pH values were observed for the control samples followed by air packaged salted samples. In both samples pH values increased progressively during the storage period. In contrast lower pH values were recorded for MAP packaged samples with or without oregano oil. The increase of pH values during the storage period may be attributed to the production of basic compounds such as ammonia, trimethylamine as well as other biogenic amines by fish spoilage bacteria (Boskou & Debevere, 2000; Kyrana et al., 1997; Masniyom et al., 2002; Ruiz-Capillas & Moral, 2001).

The above reported pH values for the control samples are in agreement with those reported by Kyrana et al. (1997) and Tejada and Huidobro (2002) for sea bream fillets stored in melting ice.

3.3. Determination of total volatile basic nitrogen

TVBN values for all sea bream packaged fillets are presented in Table 2. The TVBN maybe considered as a quality index for fish. Its increase is related to the activity of spoilage bacteria and endogenous enzymes (Kyrana et al., 1997; Ozogul et al., 2004; Ruiz-Capillas & Moral, 2005; Vareltzis et al., 1997). The action of such enzymes results in the formation of compounds including ammonia, monoethylamine, dimethylamine as well as trimethylamine (Debevere & Boskou, 1996) imparting characteristic off-flavours to fish.

The initial (day 0) value of 15.9 mg N/100 g is similar or lower than the values reported for fresh sea bream by other authors: 20 mg N/100 g after 1 day of storage at 2 ± 1 °C (Tejada & Huidobro, 2002); 25.3 mg N/100 g after 3 days

Table 2
Total volatile basic nitrogen[A](TVBN) (mg N/100 g) of sea bream stored at 4 ± 0.5 °C

Storage time[B](days)	Packaging conditions				
	Air packaged (control) (A1)	Air packaged salted (A2)	MAP salted (M1)	MAP salted 0.4% oregano oil (M2)	MAP salted 0.8% oregano oil (M3)
0	15.9 ± 0.4[a]	15.9 ± 0.4[a]	15.9 ± 0.4[a]	15.9 ± 0.4[a]	15.9 ± 0.4[a]
4	22.0 ± 0.6[a]	18.2 ± 0.2[b,c]	19.0 ± 0.3[b]	17.7 ± 0.3[c]	17.9 ± 0.4[c]
8	25.4 ± 0.3[a]	20.3 ± 0.3[b]	20.6 ± 0.5[b]	18.2 ± 0.2[c]	18.7 ± 0.5[c]
12	31.5 ± 0.7[a]	22.9 ± 0.5[b]	21.3 ± 0.6[c]	19.5 ± 0.4[d]	19.8 ± 0.4[d]
16	36.1 ± 0.5[a]	30.8 ± 0.8[b]	23.6 ± 0.3[c]	22.0 ± 0.5[d]	22.5 ± 0.2[d]
20	40.6 ± 0.4[a]	35.3 ± 0.7[b]	27.7 ± 0.2[c]	26.2 ± 0.3[d]	26.1 ± 0.4[d]
24	47.6 ± 0.3[a]	39.8 ± 0.6[b]	33.8 ± 0.5[c]	28.2 ± 0.7[d]	27.7 ± 0.3[d]
28	58.4 ± 0.9[a]	45.3 ± 0.4[b]	36.6 ± 0.7[c]	33.5 ± 0.6[d]	29.4 ± 0.7[e]
33	71.8 ± 1.2[a]	49.9 ± 0.6[b]	45.7 ± 0.4[c]	40.5 ± 0.3[d]	35.2 ± 0.9[e]

[a–e] Values in the same line followed by a 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 non-treated product.

of storage at 4 °C (Chouliara, Savvaidis, Panagiotakis, & Kontominas, 2004) and 26.0 mg N/100 g at 0 days (Kyrana et al., 1997).

Of course, variation in TVBN values of a particular fish species is related to the fish non-protein nitrogen content, which in turn depends on type of fish feeding, season of catching, fish size as well as other environmental factors. Lastly it is directly related to microbial activity in the fish tissue (Connell, 1990; Debevere & Boskou, 1996; Kyrana et al., 1997; Ozogul et al., 2004).

As shown in Table 2 TVBN values increased progressively with time of storage for all treatments. Increase in TVBN values followed the order: MAP/salt/oregano oil 0.8% (v/w) < MAP/salt/oregano oil 0.4% (v/w) < MAP/salt < air/salt < air.

The TVBN values of control (air packaged) samples were significantly higher ($p < 0.05$) than those of air packaged salted samples throughout the entire storage period. Such differences may be attributed to the preservative effect of salt (Goulas & Kontominas, 2005).

MAP also produced an obvious preservative effect lowering significantly ($p < 0.05$) the TVBN values of sea bream fillets as compared with salted samples packaged in air. This effect can be attributed mainly to CO_2, owing to its bacteriostatic properties (Farber, 1991; Sivertsvik et al., 2002).

The significantly ($p < 0.05$) lower TVBN values of two groups of samples containing oregano EO may be attributed to the antibacterial properties of oregano EO and more specifically to its phenolic constituents: carvacrol and thymol (Baydar et al., 2004; Burt, 2004; Mahmoud et al., 2004). Given the hydrophobic nature of these phenolic compounds, they are dissolved in the lipid phase of fish fillets interfering with the phospholipid bilayer of the cell membrane, which causes an increase in the permeability and a loss of cellular constituents (Burt, 2004; Mahmoud et al., 2004; Mejlholm & Dalgaard, 2002).

Another possible route for exertion of phenolic compounds antimicrobial activity is the impairment of a variety of enzyme systems and inactivation or destruction of genetic material (Mahmoud et al., 2004).

TVBN values of the control samples reached the value of 71.8 mg N/100 g after 33 days of storage, exceeding the upper acceptability limit set by the EU (EEC, 1995) for TVBN values of fish (35 mg N/100 g of fish flesh) after ca. 15–16 days of storage. The TVBN values of air packaged salted samples was exceeded this limit after ca. 20 days, while the TVBN values of salted in modified atmosphere packaged samples after ca. 26–27 days. Finally, the two groups of sea bream salted fillets stored in MAP containing oregano EO (0.4% and 0.8% v/w) exceeded the acceptability limit after ca. 29–30 and 33 days, respectively.

Based on the TVBN value of 35 mg N/100 g of fish flesh it may be postulated that light salting extended the shelf-life of fresh sea bream by ca. 4–5 days, salting plus MAP by ca. 11–12 days, salting plus MAP and 0.4% oregano EO by ca. 14–15 days and lastly salting plus MAP and 0.8% oregano EO by ca. 17–18 days.

It is interesting to note that up to 24 days of storage there were no statistically significant differences ($p < 0.05$) in TVBN values between the two MAP sample lots containing 0.4% and 0.8% v/w oregano EO, respectively. This may be attributed to the fact that when the total viable count (TVC) of fish flesh is still relatively low, a 0.4% (v/w) concentration of oregano EO in combination with MAP and light salting is adequate to inhibit the microbial activity as exhibited by low TVBN values (Boskou & Debevere, 2000; Connell, 1990). When microbial populations increase, a higher concentration of antibacterial agent is required to inhibit the microbial activity.

To our knowledge very little information is available in the literature on the effect of essential oils on the TVBN values of fish. As reported by Mahmoud et al. (2004), dipping of carp (*Cyprinus carpio*) fillets in a solution containing 0.5% carvacrol and 0.5% thymol following by storage at 5 °C produced fillets which reached a TVBN value of 30 mg N/100 g of fish flesh after 12 days of storage whereas the control reached this value after only 4 days.

Table 3
Trimethylamine nitrogen[A](TMAN) (mg N/100 g) of sea bream stored at 4 ± 0.5 °C

Storage time[B](days)	Packaging conditions				
	Air packaged (control) (A1)	Air packaged salted (A2)	MAP salted (M1)	MAP salted 0.4% oregano oil (M2)	MAP salted 0.8% oregano oil (M3)
0	0.31 ± 0.02^{a}	0.31 ± 0.02^{a}	0.31 ± 0.02^{a}	0.31 ± 0.02^{a}	0.31 ± 0.02^{a}
4	0.46 ± 0.04^{a}	0.45 ± 0.02^{a}	0.49 ± 0.05^{a}	0.36 ± 0.04^{a}	0.37 ± 0.05^{a}
8	0.70 ± 0.05^{a}	0.48 ± 0.04^{b}	0.52 ± 0.03^{b}	0.51 ± 0.04^{b}	0.43 ± 0.06^{b}
12	1.12 ± 0.05^{a}	0.68 ± 0.04^{b}	0.65 ± 0.05^{b}	0.46 ± 0.03^{c}	0.50 ± 0.05^{c}
16	1.86 ± 0.10^{a}	1.02 ± 0.07^{b}	0.79 ± 0.06^{c}	$0.69 \pm 0.04^{c,d}$	0.62 ± 0.03^{d}
20	2.84 ± 0.22^{a}	1.78 ± 0.12^{b}	1.19 ± 0.07^{c}	0.87 ± 0.06^{d}	0.92 ± 0.05^{d}
24	3.71 ± 0.18^{a}	2.61 ± 0.21^{b}	1.54 ± 0.10^{c}	1.26 ± 0.07^{d}	1.29 ± 0.08^{d}
28	5.25 ± 0.53^{a}	3.41 ± 0.14^{b}	2.39 ± 0.20^{c}	$2.12 \pm 0.08^{c,d}$	1.82 ± 0.25^{d}
33	7.38 ± 0.30^{a}	5.02 ± 0.33^{b}	4.29 ± 0.19^{c}	3.74 ± 0.25^{d}	3.25 ± 0.12^{e}

[a–e] Values in the same line followed by a 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 non-treated product.

3.4. Determination of trimethylamine nitrogen

TMAN values of sea bream fillets are presented in Table 3. TMAN content is often used as a biochemical index to assess keeping quality and shelf-life of fish (Connell, 1990). In marine fish, as is sea bream, TMA which is formed from trimethylamine oxide (TMAO) as a result of bacterial enzyme activity, is the main component responsible for an unpleasant "fishy" odour (Connell, 1990; Debevere & Boskou, 1996; Shakila, Vijayalakshmi, & Jeyasekaran, 2003; Sivertsvik et al., 2002).

The initial TMAN content of sea bream fillets was low (0.31 mg N/100 g of flesh) indicating the freshness of the samples. This value is in good agreement with that of 0.4 mg N/100 g reported by Tejada and Huidobro (2002) for the initial TMAN content of sea bream.

The TMAN content of salted sea bream samples packaged in air was significantly lower ($p < 0.05$) than TMAN content of unsalted fish samples packaged in air after day 4 of storage. This observation is in agreement with our previous results (Goulas & Kontominas, 2005) for chub mackerel fillets and is indicative of the preservative effect of salt. This effect is due to the decrease in water activity (a_w) and thus prevention of growth of many spoilage microorganisms (Horner, 1997; Leroi, Joffraud, & Chevalier, 2000).

After ca. day 8 of storage TMAN values of the control samples were significantly ($p < 0.05$) higher and increased with a significantly higher rate than the corresponding values of all other groups of samples throughout storage period. TMAN reached the value of 7.38 mg N/100 g after 33 days of storage. Significantly lower ($p < 0.05$) were the final TMAN values for all other lots of sea bream fillets (3.25–5.02 mg N/100 g) indicating the preservative effect of salt, MAP as well as oregano oil. The preservative effect of MAP on sea bream fillets is apparent. Data in Table 3 show that TMAN values recorded in MAP salted sea bream fillets were significantly lower ($p < 0.05$) than those of salted sea bream samples packaged in air.

This can be attributed to the inhibitory action of CO_2 after dissolution in both the aqueous and fatty phase of fish (Debevere & Boskou, 1996; Farber, 1991; Goulas et al., 2005; Masniyom et al., 2002; Ozogul et al., 2004; Ruiz-Capillas & Moral, 2001; Sivertsvik et al., 2002). The bacteriostatic effect of CO_2 can be attributed to the alteration of cell membranes, inhibition of enzymes, changes in the physicochemical properties of proteins and intracellular pH changes (Farber, 1991; Sivertsvik et al., 2002).

The lower TMAN values of the MAP and oregano EO treated samples in comparison with the MAP treated samples may be attributed to antibacterial properties of phenolic compounds of oregano EO as above reported for TVBN values.

A wide range of TMAN values have been reported by various authors as acceptability limit: 1 mg N/100 g (Kyrana et al., 1997; Tejada & Huidobro, 2002); 5 mg N/100 g (Masniyom et al., 2002); 5–10 mg N/100 g (Ozogul et al., 2004); 12 mg N/100 g (Ruiz-Capillas & Moral, 2005); 10–15 mg N/100 g (Connell, 1990).

The great variation in the acceptability level can be attributed to the fact that TMAN values vary with species, season, storage conditions as well as bacterial and intrinsic enzymes activity (Connell, 1990; Debevere & Boskou, 1996; Sivertsvik et al., 2002).

As reported by Shakila et al. (2003), changes in TVBN and TMAN values of six commercial fish species studied at ambient temperature greatly varied although experimental conditions were identical for all fish species.

Given the great variation in the reported TMAN acceptability limits for various fish species, the TMAN values reported in the Table 3 as well as the odour scores of raw sea bream samples (Table 5), a more realistic TMAN limit for sea bream fillets of ca. 2–3 mg N/100 g may be proposed.

Present TMAN values are significantly lower than the general TMAN limit for fish reported by Connell (1990) (10–15 mg N/100 g) but are in agreement with the low TMAN levels (1.8–4.8 mg N/100 g flesh) at the rejection point reported for other Sparidae species (Civera, Turi, Parisi, & Fazio, 1995). They are also in agreement with low TMAN values for sea bream at the rejection point (2.0–8.87 mg N/100 g) reported by Chouliara et al. (2004), Kyrana et al. (1997) and Tejada and Huidobro (2002).

3.5. Determination of 2-thiobarbituric acid

The TBA value is an index of lipid oxidation measuring malondialdehyde (MDA) content. MDA formed through hydroperoxides, which are the initial reaction product of polyunsaturated fatty acids with oxygen (Fernandez, Perez-Alvarez, & Fernandez-Lopez, 1997).

TBA values for sea bream samples are presented in Table 4. Generally, TBA values of salted samples packaged in air were significantly higher ($p < 0.05$) than those of non-salted packaged in air (control) samples.

This observation is indicative of the fact that salting clearly accelerates lipid oxidation and considerably reduces the oxidative stability of sea bream lipids and is in agreement with the results reported previously (Aubourg & Ugliano, 2002; Guillen & Ruiz, 2004; Goulas & Kontominas, 2005; Ruiz-Capillas & Moral, 2001). The mean value of salt content of fillet samples in the present study was 1.9 ± 0.3 g NaCl/100 g of flesh, at day 0 immediately after the brining treatment.

A statistically significant difference ($p < 0.05$) was observed in TBA values of the two MAP samples containing 0.4% and 0.8% oregano EO as compared to MAP salted samples indicating the strong antioxidant effect of oregano EO which acts as a radical scavenger (Kulisic et al., 2004; Skerget et al., 2005; Tsimidou et al., 1995; Vekiari et al., 1993; Wong & Kitts, 2002).

It is evident that no significant differences ($p < 0.05$) were observed between the two lots of samples containing

Table 4
Thiobarbituric acid[A] (TBA) (mg of malondialdehyde/kg) of sea bream stored at 4 ± 0.5 °C

Storage time[B] (days)	Packaging conditions				
	Air packaged (control) (A1)	Air packaged salted (A2)	MAP salted (M1)	MAP salted 0.4% oregano oil (M2)	MAP salted 0.8% oregano oil (M3)
0	0.18 ± 0.04^{a}	0.18 ± 0.04^{a}	0.18 ± 0.04^{a}	0.18 ± 0.04^{a}	0.18 ± 0.04^{a}
4	0.20 ± 0.05^{a}	0.31 ± 0.06^{a}	$0.38 \pm 0.10^{a,b}$	0.09 ± 0.02^{c}	0.15 ± 0.02^{c}
8	0.24 ± 0.06^{a}	0.48 ± 0.05^{b}	0.73 ± 0.06^{c}	0.21 ± 0.03^{a}	0.22 ± 0.05^{a}
12	0.40 ± 0.07^{a}	0.58 ± 0.09^{b}	0.90 ± 0.16^{c}	0.25 ± 0.07^{d}	0.28 ± 0.05^{d}
16	0.52 ± 0.05^{a}	0.65 ± 0.06^{a}	0.82 ± 0.07^{b}	0.31 ± 0.05^{c}	0.22 ± 0.04^{c}
20	0.72 ± 0.06^{a}	0.77 ± 0.10^{a}	0.73 ± 0.08^{a}	0.29 ± 0.05^{b}	0.20 ± 0.03^{b}
24	0.91 ± 0.08^{a}	1.12 ± 0.07^{b}	$0.98 \pm 0.09^{a,b}$	0.48 ± 0.07^{c}	0.38 ± 0.09^{c}
28	0.81 ± 0.09^{a}	0.89 ± 0.12^{a}	1.60 ± 0.20^{b}	0.39 ± 0.11^{c}	0.40 ± 0.12^{c}
33	0.72 ± 0.05^{a}	0.99 ± 0.10^{b}	1.31 ± 0.16^{c}	0.44 ± 0.05^{d}	0.30 ± 0.07^{e}

[a–e] Values in the same line followed by a 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 non-treated product.

oregano oil up to 28 days of storage while after day 33 oregano oil 0.8% appeared to be slightly more effective. From a practical point of view this effect of oregano EO is indicative that the 0.4% v/w percent of EO is an effective antioxidant for salted MA packaged fish samples.

The above reported results are in good agreement with those of Tsimidou et al. (1995), for antioxidant activity of 0.5 and 1% v/w oregano EO in mackerel oil at 40 °C.

As can be seen in Table 4, TBA values in most cases increased gradually up to a certain point during storage; followed by either a decrease in values or a lower increase rate. Given that TBA value is a measurement of MDA content, decrease in MDA may be caused by interaction between MDA and amino acids, proteins, glucose and other fish constituents (Fernandez et al., 1997). This observation is in agreement with previous reports (Curzio & Quaranta, 1982; Fernandez et al., 1997; Goulas et al., 2005; Ruiz-Capillas & Moral, 2001).

According to Connell (1990), 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/taste. The TBA values of the present sea bream samples exceeded the value of 1 mg MDA/kg only for air packaged salted samples (1.12 mg MDA/kg) and MAP salted samples (1.31 and 1.60 mg MDA/kg).

3.6. Sensory evaluation

The results of the sensory evaluation of raw sea bream fillet samples are presented in Table 5. The concentrations of oregano EO used produced a distinct but organoleptically acceptable pleasant odour in raw fish fillets.

As the results show, up to 15–16 days of storage all sea bream packaged samples received an odour score significantly higher or similar (control samples) than the lower acceptability limit of 6. Salted samples reached this limit

Table 5
Sensory evaluation[A,B] of raw sea bream stored at 4 ± 0.5 °C

Sensory parameter	Packaging conditions	Storage time[C] (days)								
		0	4	8	12	16	20	24	28	33
Odour	A1	10.0 ± 0.0^{a}	9.8 ± 0.2^{a}	9.1 ± 0.3^{b}	7.8 ± 0.3^{c}	5.9 ± 0.5^{d}	3.5 ± 0.5^{e}	1.5 ± 0.5^{f}	0.0 ± 0.0^{g}	0.0 ± 0.0^{g}
	A2	10.0 ± 0.0^{a}	9.5 ± 0.2^{b}	$9.2 \pm 0.2^{b,c}$	8.8 ± 0.2^{c}	6.7 ± 0.5^{d}	6.2 ± 0.8^{d}	4.8 ± 0.5^{e}	4.1 ± 0.5^{f}	2.5 ± 0.2^{g}
	M1	10.0 ± 0.0^{a}	9.3 ± 0.3^{b}	8.8 ± 0.4^{b}	$8.5 \pm 0.3^{b,c}$	$8.2 \pm 0.4^{b,c}$	7.7 ± 0.3^{c}	$7.0 \pm 0.9^{c,d}$	5.7 ± 0.6^{e}	4.7 ± 0.7^{e}
	M2	10.0 ± 0.0^{a}	10.0 ± 0.0^{a}	10.0 ± 0.0^{a}	10.0 ± 0.0^{a}	10.0 ± 0.0^{a}	9.4 ± 0.5^{b}	9.1 ± 0.3^{b}	7.2 ± 0.3^{c}	5.5 ± 0.5^{d}
	M3	10.0 ± 0.0^{a}	10.0 ± 0.0^{a}	10.0 ± 0.0^{a}	10.0 ± 0.0^{a}	10.0 ± 0.0^{a}	9.7 ± 0.3^{a}	9.5 ± 0.2^{b}	8.1 ± 0.5^{c}	6.9 ± 0.3^{d}
Flesh texture	A1	10.0 ± 0.0^{a}	10.0 ± 0.0^{a}	9.3 ± 0.2^{b}	8.6 ± 0.5^{b}	7.2 ± 0.6^{c}	6.1 ± 0.5^{c}	3.5 ± 0.6^{d}	0.0 ± 0.0^{e}	0.0 ± 0.0^{e}
	A2	10.0 ± 0.0^{a}	10.0 ± 0.0^{a}	10.0 ± 0.0^{a}	9.0 ± 0.2^{b}	7.9 ± 0.5^{c}	$6.9 \pm 0.8^{c,d}$	$7.0 \pm 1.0^{c,d}$	6.4 ± 0.5^{d}	2.0 ± 0.4^{e}
	M1	10.0 ± 0.0^{a}	10.0 ± 0.0^{a}	9.8 ± 0.2^{a}	8.4 ± 0.3^{b}	7.8 ± 0.2^{b}	6.4 ± 0.4^{c}	$5.5 \pm 0.7^{c,d}$	4.5 ± 0.6^{d}	2.5 ± 0.5^{e}
	M2	10.0 ± 0.0^{a}	10.0 ± 0.0^{a}	10.0 ± 0.0^{a}	9.8 ± 0.1^{a}	8.9 ± 0.3^{b}	8.8 ± 0.6^{b}	8.1 ± 0.6^{b}	7.8 ± 0.7^{b}	5.6 ± 0.4^{c}
	M3	10.0 ± 0.0^{a}	10.0 ± 0.0^{a}	10.0 ± 0.0^{a}	9.8 ± 0.1^{a}	9.8 ± 0.2^{a}	9.5 ± 0.3^{a}	8.6 ± 0.5^{b}	$7.5 \pm 0.8^{b,c}$	6.7 ± 0.6^{c}
Flesh colour	A1	10.0 ± 0.0^{a}	10.0 ± 0.0^{a}	9.8 ± 0.2^{a}	8.8 ± 0.4^{b}	7.9 ± 0.3^{c}	6.4 ± 0.8^{d}	5.1 ± 0.4^{e}	$4.5 \pm 0.7^{e,f}$	3.5 ± 0.6^{f}
	A2	10.0 ± 0.0^{a}	10.0 ± 0.0^{a}	10.0 ± 0.0^{a}	8.1 ± 0.6^{b}	$7.4 \pm 0.8^{b,c}$	$6.3 \pm 0.9^{c,d}$	5.5 ± 0.8^{d}	4.8 ± 0.8^{d}	3.0 ± 0.4^{e}
	M1	10.0 ± 0.0^{a}	9.5 ± 0.1^{b}	9.5 ± 0.3^{b}	8.2 ± 0.5^{c}	7.6 ± 0.7^{c}	7.0 ± 0.3^{c}	5.7 ± 0.5^{d}	4.1 ± 0.6^{e}	3.5 ± 0.5^{e}
	M2	10.0 ± 0.0^{a}	9.0 ± 0.2^{b}	$9.2 \pm 0.5^{b,c}$	$9.0 \pm 0.3^{b,c}$	8.3 ± 0.4^{c}	7.3 ± 0.4^{d}	6.5 ± 0.7^{d}	6.0 ± 0.8^{d}	4.5 ± 0.6^{e}
	M3	10.0 ± 0.0^{a}	9.0 ± 0.3^{b}	8.3 ± 0.4^{b}	$7.9 \pm 0.4^{b,c}$	$7.8 \pm 0.3^{b,c}$	$7.0 \pm 0.6^{c,d}$	$6.8 \pm 0.5^{c,d}$	$7.0 \pm 0.9^{c,d}$	6.2 ± 0.7^{d}

[a–g] Values in the same line followed by a different letter are significantly different ($p < 0.05$).
[A] Scale from 10 to 0 (10 excellent and 0 very bad). Acceptability limit 6.
[B] Values represent the mean of six determinations ($n = 2 \times 3$) ± SD.
[C] Values on day 0 correspond to non-treated product.

after 20–21 days, MAP salted samples after 27–28 days, MAP salted samples containing 0.4% oregano EO after ca. 32–33 days, while MAP salted samples containing 0.8% oregano EO never reached this limit throughout the entire storage period.

On the other hand up to ca. 20–21 days of storage the flesh texture and flesh colour of all packaged samples received scores above the acceptability limit of 6. Obviously, the most sensitive sensory attribute for quality evaluation of sea bream was odour.

The shelf-life of ca. 32–33 days for MAP salted samples with 0.4% oregano EO corresponds to a ca. 5 days extension in comparison with MAP salted samples while even greater shelf-life extension was achieved with 0.8% oregano oil. Also, MAP extended the shelf-life of salted samples ca. 7 days as compared with the salted samples and ca. 12 days as compared with the control samples.

Consequently, the total shelf-life extension, which was achieved in the present study between control and salted MAP sea bream fillets with 0.4% oregano oil, was ca. 17 days while even a greater shelf-life extension was achieved with 0.8% oregano oil.

From the above results it is obvious that oregano EO substantially contributes to the extension of shelf-life of MAP packaged salted sea bream fillets delaying spoilage while imparting a pleasant flavour to fish products.

An interesting observation is that the flesh texture scores of control and salted packaged in the presence of air samples showed a slower decreasing rate than the odour scores of the same samples, while on the contrary, the flesh texture and flesh colour scores of MAP salted with or without oregano EO samples showed a faster decreasing rate than the odour scores.

This observation may be attributed to the drip loss observed mainly in the MAP with or without oregano EO fillet samples (Table 6). The phenomenon of the excessive drip loss of fish muscle when packaged in modified atmospheres results from a graduate loss in ability of fish proteins to retain water as the storage time progresses and has been also observed by other authors (Boknaes et al., 2002; Masniyom et al., 2002; Pastoriza et al., 1998; Ruiz-Capillas & Moral, 2001).

As reported by Masniyom et al. (2002) and Dalgaard et al. (1993), the higher the content of CO_2 in MAP the higher the drip loss. This may be due to a greater loss of the water-holding capacity of muscle protein at lower pH values produced from the dissolution of CO_2 in the aqueous phase of fish muscle (Pastoriza et al., 1998; Sivertsvik et al., 2002). The relatively low storage temperature used in the present experiment favors the dissolution of CO_2 in both the aqueous and fatty phase of fish given that the lower the temperature, the higher the solubility of CO_2 (Siverstvik et al., 2002).

The flesh colour acceptability scores of all packaged fillet samples decreased gradually with the time of refrigerated storage with a relatively similar decrease rate as the texture scores.

As the results in Tables 2, 3 and 5 show, there is a good correlation between sensory scores and biochemical indices of quality determined. Odour proved to be the most sensitive of the sensory properties evaluated. Based on TVBN values and given that the upper acceptability limit for TVBN is the value of 35 mg N/100 g (Connell, 1990) the shelf-life achieved was ca. 15–16 days for the control, ca. 20–21 days for salted air packaged, ca. 26–27 days for salted MAP samples, ca. 29–30 days for 0.4% oregano EO salted MAP samples and ca. 33 days for 0.8% oregano EO salted MAP fillet samples, respectively. These values are in good agreement with the values above reported for shelf-life based on odour scores. Similarly there was good correlation between shelf-life based on odour scores and TMAN values (Tables 3 and 5).

According to Pastoriza et al. (1998), the shelf-life of hake (*Merluccius merluccius*) slices was extended by 8 days upon dipping in NaCl solution (5% w/v) for 5 min prior to MAP (50% CO_2/45% N_2/5% O_2) storage at 2 ± 1 °C. This shelf-life extension is in agreement with our shelf-life extension in MAP salted samples (ca. 12 days) based on odour scores given the differences in time and concentration of NaCl solution, which we used.

Mejlholm and Dalgaard (2002), reported that the oregano oil (0.05% v/w) extended the shelf-life of naturally contaminated MAP cod (*Gadus morhua*) fillets from 11–12 days to 21–26 days at 2 °C. However, this is a significantly higher shelf-life extension than the 5 days which was achieved in the present study for MAP sea bass fillets after the 0.4% v/w oregano EO addition.

Mahmoud et al. (2004), reported that the dipping treatment of carp (*Cyprinus carpio*) fillets in 1% (carvacrol and thymol) mixture extended the shelf-life of the product from 4 to 12 days at 5 °C.

According to Harpaz et al. (2003), the addition of oregano and thyme EO at 0.05% v/v can considerably slow down the process of spoilage of Asian sea bream (*Lates calcarifer*) and the fish treated with above concentration of EO were still fit for human consumption after 33 days of storage at 0–2 °C.

Table 6
Drip loss[A] (%) of sea bream fillet samples as a percentage of initial fillet weight after 33 days of storage at 4 ± 0.5 °C

Packaging conditions				
Air packaged (control) (A1)	Air packaged salted (A2)	MAP salted (M1)	MAP salted 0.4% oregano oil (M2)	MAP salted 0.8% oregano oil (M3)
2.8 ± 0.9^{a}	1.2 ± 0.7^{b}	7.0 ± 1.4^{c}	5.5 ± 0.9^{c}	6.1 ± 1.1^{c}

[A] Values represent the mean of six determinations ($n = 2 \times 3$) ± SD.

Finally Tassou et al. (1996), reported that the treatment of sea bream (*Sparus aurata*) with olive oil, lemon juice and oregano followed by storage under MAP of 40% CO_2/30% O_2/30% N_2 at 0 ± 1 °C showed both bacteriostatic and bactericidal effect on *Staphylococcus aureus* and *Salmonella enteritis* inoculated onto the substrate as well as on the autochthonous flora.

From a practical point of view the use of oregano essential oil for fish preservation is advantageous since besides contributing to the extension of fish shelf-life in addition it contributes to the development of a pleasant odour and taste favourable to consumers, given that the flavour of roasted and baked fish is compatible with that of oregano in the Mediterranean area.

4. Conclusions

Based primarily on sensory, but also on biochemical indices determination oregano essential oil in combination with MAP and light salting was the most effective treatment for the preservation of sea bream fillets followed by MAP.

Based on odour scores, the combination of 0.8% oregano EO, MAP and light salting extended the shelf-life of sea bream fillets under refrigeration by more than 17 days, followed by the combination of 0.4% oregano EO, MAP and light salting (shelf-life extension by ca. 17 days) MAP and light salting (ca. 12 days), light salting (ca. 5 days) as compared to control samples (shelf-life ca. 15–16 days).

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