Food Chemistry 115 (2009) 169–175

Contents lists available at ScienceDirect

Food Chemistry

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

Effect of thyme essential oil and packaging treatments on fresh Mediterranean swordfish fillets during storage at 4 °C

S. Kykkidou [a], V. Giatrakou [a], A. Papavergou [b], M.G. Kontominas [a], I.N. Savvaidis [a,*]

[a] University of Ioannina, Department of Chemistry, Laboratory of Food Chemistry and Food Microbiology, Ioannina 45110, Greece
[b] Department of Food Hygiene and Technology, Faculty of Veterinary Medicine, Aristotle University of Thessaloniki, Thessaloniki 54124, Greece

ARTICLE INFO

Article history:
Received 11 June 2008
Received in revised form 11 October 2008
Accepted 26 November 2008

Keywords:
Swordfish
Packaging
Thyme oil, essential oils, natural antimicrobials
Shelf-life

ABSTRACT

The present study evaluated the effect of thyme essential oil and packaging on fresh Mediterranean swordfish fillets during storage at 4 °C. Treatments in the present study included the following: air (A), modified atmosphere packaging (M), air with thyme oil (AT) and MAP with thyme oil (MT). Of the physicochemical parameters examined, TBA values for A and M swordfish samples were variable, indicative of no specific oxidative rancidity trend, whereas MT treatment inhibited lipid oxidation in swordfish samples during storage. On the basis of microbiological and sensory data, TMA-N and TVB-N limit values of acceptability for Mediterranean swordfish, of ca. 3.72 and 24.5 mg N/100 g, for the initiation of fresh Mediterranean swordfish spoilage, may be proposed. Of the treatments used in the present study, MT and M were the most effective for the inhibition of pseudomonads and H_2S-producing bacteria in swordfish. Lactic acid bacteria and *Enterobacteriaceae* (to a lesser extent) were also found to be part of the natural microbial flora of swordfish, irrespective of packaging treatment. Based primarily on sensory data, the shelf-lives of fresh refrigerated Mediterranean swordfish were 8 and 13 days under aerobic and MAP conditions, respectively. Addition of 0.1% thyme essential oil extended the product's shelf-life under aerobic conditions by 5 days, whereas the combination of MAP and thyme oil resulted in a significant shelf-life extension of the swordfish fillets, i.e. by approximately 7½ days, according to sensory data, as compared to the control sample.

1. Introduction

Fresh seafood has a short shelf-life, which causes substantial practical problems for its distribution. Improvements in the shelf-life of a product can have an important economic feedback by reducing losses attributed to spoilage and by allowing the products to reach distant and new markets (Rhodehamel, 1992).

Swordfish (*Xiphias gladius*) is one of the pelagic fish species belonging to the family *Xiphidae*, mainly caught in the Atlantic, Pacific and Indian oceans and temperate waters of the Mediterranean sea. Swordfish is a fish of great consumption worldwide, due to its low fat content and high protein content. It is commonly sold for immediate consumption at retail stores as filleted fish (steaks), whilst ice storage has been widely used to prolong its shelf-life. Swordfish is a perishable food, due to bacteriological and enzymatic activities which take place after death.

Modified atmosphere packaging (MAP), in combination with refrigeration, has proven to be an effective preservation method for the extension of shelf-life of fresh fish and fish products (Sivertsvik, Jeksrud, & Rosnes, 2002; Stammen, Gerdes, & Caporaso, 1990). Whilst abundant data exist on preservation of marine fish from various waters (tropical, temperate and Mediterranean) using MAP, including cod (Dalgaard, Gram, & Huss, 1993), haddock (Dhananjaya & Stroud, 1994), herring (Özogul, Taylor, Quantic, & Özogul, 2000), sardine (Özogul, Polat, & Özogul, 2004), salmon (Hoz, Lopez-Galvez, Fernandez, Hierro, & Ordonez, 2000), hake (Ruiz-Capillas & Moral, 2001), sea bass (Masniyom, Benjakul, & Visessanguan, 2002) and mullet (Pournis, Papavergou, Badeka, Kontominas, & Savvaidis, 2005), there is limited information in the literature on the effects of MAP/vacuum on preservation of fresh swordfish (Lannelongue, Finne, Hanna, Nickelson, & Vanderzant, 1982; Oberlender, Hanna, Miget, Vanderzant, & Finne, 1983). A common feature of these studies is that no sensory evaluation was carried out which is the main evaluation criterion for fresh fish quality.

In one of these aforementioned studies, swordfish (*X. gladius*) steaks were held in retail packages containing 100% CO_2 and in mixtures of 40% and 70% CO_2 in combination with oxygen or nitrogen. It was shown that the inhibitory effect of CO_2 on psychrotrophic, aerobic Gram-negative spoilage bacteria was proportional to the CO_2 concentration and was higher in atmospheres of CO_2:N_2 than in CO_2:O_2. Swordfish steaks stored in CO_2-enriched atmospheres had lower total volatile basic nitrogen (TVB-N), trimethylamine nitrogen (TMA-N) and total volatile acid

* Corresponding author. Tel.: +30 2651098343; fax: +30 2651098795.
E-mail address: isavvaid@uoi.gr (I.N. Savvaidis).

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doi:10.1016/j.foodchem.2008.11.083

(TVA) than had steaks stored in air (Lannelongue et al., 1982). In a related study, conducted by these authors, a flow-through controlled atmosphere packaging system, using different CO_2-enriched gaseous compositions, was demonstrated to be effective in retarding the growth of microorganisms on fresh swordfish steaks held at 2 °C for 22 days. Of the chemical indicators, both TVB-N and TMA-N increased more slowly for swordfish steaks stored in CO_2-enriched atmospheres than for steaks stored in air (Oberlender et al., 1983). Recently, vacuum packaging (VP) extended the shelf-life of liquid-smoked swordfish slices (that had been kept for 5 months at −20 °C) by ca. 30 days as compared to MAP (5% O_2, 45% CO_2 and 50% N_2) (Muratore & Licciardello, 2005).

Essential oils (EOs), such as thyme and oregano, have natural antimicrobial and antioxidant properties with the potential to extend the shelf-life of foods. Thymol and carvacrol are the most active constituents of thyme essential oils (EOs) with a wide spectrum of antimicrobial and antioxidant properties (Burt, 2004; Lambert, Skandamis, Coote, & Nychas, 2001). Among the EOs from various aromatic plants, thyme oil has increasingly gained the interest of research groups as a potential natural antimicrobial and antioxidant agent. The antimicrobial and antioxidant activities of thyme essential oil (EO) have been studied "in vitro", using various model foods (Burt, 2004; Holley & Patel, 2005), as well as commercial food products, such as seafood (Goulas & Kontominas 2007; Harpaz, Glatman, Drabkin, & Gelman, 2003; Mejlhom & Dalgaard, 2002) beef (Skandamis, Tsigarida, & Nychas, 2002), pork (Ismaiel & Pierson, 1990) etc. Limited data, however, are available with regard to the application of EOs, including thyme oil, for extension of shelf-life of seafood. Thus, the aim of the present work was to (i) determine the physicochemical, bacteriological and sensory changes of refrigerated Mediterranean swordfish, using either thyme oil or packaging (aerobic, MAP) and/or their combination and (ii) study the effects of thyme oil, packaging and their combination on the shelf-life of fresh Mediterranean swordfish (fillets) during storage at 4 °C.

2. Materials and methods

2.1. Sample preparation

Fresh whole swordfish (*X. gladius*), caught during the period of May–June, 2007 in the south Aegean sea, of approximate weight 20–25 kg, were immediately deheaded, gutted, cleaned and put on ice. After 48 h on board a fishing vessel, the fish were landed to the fish monger market of Thessaloniki, northern Greece, repacked on ice in insulated polystyrene boxes and delivered to the laboratory within less than 24 h of landing. Immediately after delivery, whole fish were filleted (ca. 150 ± 15 g) manually. Swordfish filleted samples, except those kept on ice, were packed in low density polyethylene/polyamide (LDPE/PA/LDPE) barrier pouches, 75 μm in thickness having an oxygen permeability of 52.2 $cm^3\ m^{-2}\ d^{-1}\ atm^{-1}$ at 75% relative humidity (RH), 25 °C, and a water vapour permeability of 2.4 $g\ m^{-2}\ d^{-1}$ at 100% RH, 25 °C. Thyme essential oil (Kokkinakis S.A., Athens, Greece) was added onto the surface (two sides) of each fillet using a micropipette, so as to achieve a final 0.1% (v/w) EO concentration. Treatments included: samples in aerobic packaging (control samples A; 4 °C), samples stored under aerobic conditions with added thyme EO, 0.1% v/w (AT; 4 °C), samples stored under MAP (5% O_2/50% CO_2/45% N_2) (M; 4 °C) and finally samples under MAP (5% O_2/50% CO_2/45% N_2) with added thyme oil 0.1% v/w (MT; 4 °C). The above gas mixture (treatments M and MT) was chosen, based on information in the literature regarding storage of swordfish (Sivertsvik et al., 2002). Oxygen was used to inhibit potential growth and toxin production of *Clostridium botulinum* type E (favoured under anaerobic conditions) and to maintain the optimal appearance of the swordfish steaks. The thyme EO concentration of 0.1% v/w, applied to the swordfish fillets, was chosen on the basis of preliminary experiments (conducted in our laboratory) that established optimum EO concentration in terms of optimum sensory (odour and taste attributes evaluated) acceptability. The MAP gas mixture was prepared using a PBI-Dansensor model mix 9000 gas mixer (Ringsted, Denmark). Pouches were heat-sealed using a BOSS model N48 vacuum sealer (BOSS, Germany) connected to the gas mixer. During the entire refrigerated period (18 days) of swordfish, storage temperature was ca. 4 ± 0.5 °C and sampling was carried out at predetermined time intervals, namely: 3, 6, 8, 10, 12, 14, 16 and 18 days.

2.2. Physicochemical analysis

Trimethylamine nitrogen (TMA-N) was determined using the method of AOAC (Association of Official of Analytical Chemists, 1990). Total volatile basic nitrogen (TVB-N) was determined using the method of Malle and Poumeyrol (1989). TMA-N and TVB-N contents were expressed as mg TMA-N/100 g, or TVB-N/100 g fish muscle. TBA was determined according to the method of Pearson (1976). Thiobarbituric acid (TBA) content was expressed as mg of malondialdehyde (MDA)/kg of fish muscle. The pH value was recorded using a pH metre (Metrohm 691). Fish muscle (2 g) was homogenised thoroughly with 10 ml of distilled water and the homogenate was used for pH determination. The concentration of gases in the headspace of the package (M and MT samples) was determined using a Dansensor model checkmate 9900 headspace analyser (PBI, Denmark). A rubber septum (Systech Instr. Ltd., UK) was glued onto the surface of each pouch and pierced with a 23 gauge needle connected to the headspace analyser. The headspace analyser gave a direct reading of $\%O_2$ and $\%CO_2$ in the pouch headspace with N_2 being calculated by difference $[100\% - (\%CO_2 + \%O_2)]$.

2.3. Microbiological analysis

A sample (25 g) was taken from each fish steak, transferred aseptically into a stomacher bag (Seward Medical, UK) containing 225 ml of 0.1% peptone water), and the mixture was homogenised for 60 s using a Stomacher (Lab Blender 400) at room temperature (Seward Medical, UK). For microbial enumeration, 0.1 ml samples of serial dilutions (1:10, diluent, 0.1% peptone water) of fish homogenates were spread onto plates of various agar materials. Total viable counts (TVC) were determined using modified Long and Hammer agar after incubation for 7 days at 10 °C. Pseudomonads were enumerated on cetrimide fusidin cephaloridine agar (CFC, Oxoid code CM 559, supplemented with SR 103, Oxoid, Basingstoke, UK) and incubated at 20 °C for 2 days (Mead & Adams, 1977). Lactic acid bacteria (LAB) were enumerated on de Man Rogosa Sharpe agar (MRS, pH 6.2, Oxoid code CM361, Basingstoke, UK) and incubated at 25 °C for 4–5 days. For *Enterobacteriaceae* and H_2S-producing bacteria (including *Shewanella putrefaciens*) enumeration, a 1.0 ml sample was inoculated into 10 ml of molten (45 °C) violet red bile glucose agar (VRBGA, Oxoid code CM 485, Basingstoke, UK) and Iron Agar (IA, Oxoid code CM 867, Basingstoke, UK), respectively. After setting, an overlay of molten medium was added. For the former (VRBGA), incubation was carried out at 30 °C for 24 h. The large colonies with purple haloes were counted (Mossel, Eelderink, Koopmans, & Rossen, 1979). IA plates were incubated at 20 °C and black colonies formed by the production of H_2S were enumerated after 2–3 days. Three replicates of at least three appropriate dilutions, depending on the sampling day, were enumerated. Microbiological data were transformed into logarithms of the number of colony forming units (cfu/g). All plates

were examined visually for typical colony types and morphology characteristics associated with each growth medium.

2.4. Sensory evaluation

The attributes of cooked fish (odour and taste) were evaluated by a panel of seven experienced judges on each day of sampling. Fish samples (25 g) were cooked individually in a microwave oven at full power (1600 W), for 5 min, including defrosting time, and immediately presented to the panellists. Sensory evaluation was conducted in individual booths under controlled conditions of light, temperature and humidity. A freshly thawed fish sample, stored at −30 °C was also presented to the panellists, this serving as the master control sample. Panellists were asked to score odour and taste using a 0–10 hedonic scale with 10 corresponding to the most liked sample and zero corresponding to least liked samples (Pons-Sanchez-Cascado, Vidal-Carou, Nunes, & Veciana-Nogues, 2006). The product was defined as unacceptable (a score <5) after development of off-odours.

2.5. Statistical analysis

Experiments were replicated twice on different occasions with different fish samples. Triplicate samples were taken per replicate. Results are reported as mean values ± standard deviation (SD). Data were subjected to analysis of variance (ANOVA). The least significant difference (LSD) procedure was used to test for differences between means ($P < 0.05$) (Steel & Torrie, 1980).

3. Results

3.1. Physicochemical changes

The headspace gas atmosphere did not undergo significant changes ($P > 0.05$) in composition throughout storage time for all M and MT treatments. The concentration of CO_2 in the headspace gas atmosphere was between 45.1% and 49.8% during the entire refrigerated storage period (results not shown).

Values of pH for A, AT, M and MT swordfish samples were in the range of ca. 6.8–7.0 (results not shown) with no statistically significant differences ($P > 0.05$) between treatments.

Changes in TBA values for A, AT, M and MT swordfish samples during the 18 day storage period are shown in Fig. 1. Of all the treatments examined in the present study, MT combination treatment produced the lowest ($P < 0.05$) TBA values (<2 mg MDA/kg fish muscle) in swordfish samples during the entire refrigerated storage period, as a result of the synergistic combination of MAP and thyme essential oil.

TMA-N values of swordfish fillets are presented in Fig. 2. Low initial TMA-N content (1.18 mg N/100 g of fish muscle) indicates that the swordfish was of good quality, in agreement with the relatively low total viable counts (TVC) of 4.9 log cfu/g (Fig. 4). Production of TMA-N for AT, M and MT swordfish samples was significantly lower ($P < 0.05$) than that for the control (A) samples between days 12 and 18 of storage.

TVB-N values, for all swordfish fillets, are presented in Fig. 3. Treatments AT, M and MT produced lower ($P < 0.05$) TVB-N values than that of the control (A) samples after day 6 and until the end of the storage period.

3.2. Bacteriological changes

Changes in TVC of refrigerated swordfish fillets during storage under aerobic, MAP conditions, with or without thyme oil, are shown in Fig. 4. A, AT, M and MT swordfish samples exceeded the value of 7 log cfu/g for TVC, considered as the upper acceptability limit for fresh marine species (International Commission on Microbiological Specifications for Foods, 1978) on days 6, 9, 12 and 15 of storage, respectively. Thus, compared with the control (A) samples, a microbiological shelf-life extensions of 3, 6 and 9 days were achieved for AT, M and MT swordfish samples, respectively, as determined by TVC data (Fig. 4).

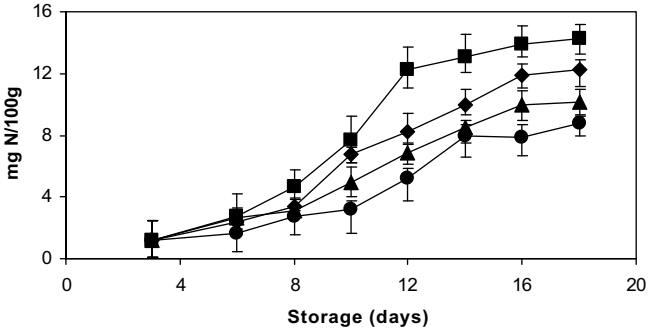

Fig. 2. Changes in trimethylamine nitrogen (TMA-N) of fresh swordfish fillets during storage at 4 °C under aerobic packaging (A; ■), aerobic packaging with thyme oil (0.1% v/w, AT; ♦), modified atmosphere packaging (M; ▲) and modified atmosphere packaging with thyme oil (0.1% v/w, MT; ●). Each point is the mean of three samples taken from two replicate experiments ($n = 3 \times 2 = 6$). Error bars show SD.

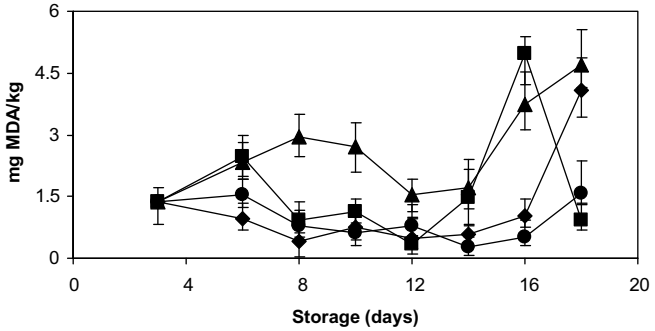

Fig. 1. Changes in thiobarbituric acid (TBA) of fresh swordfish fillets during storage at 4 °C under aerobic packaging (A; ■), aerobic packaging with thyme oil (0.1% v/w, AT; ♦), modified atmosphere packaging (M; ▲) and modified atmosphere packaging with thyme oil (0.1% v/w, MT; ●). Each point is the mean of three samples taken from two replicate experiments ($n = 3 \times 2 = 6$). Error bars show SD.

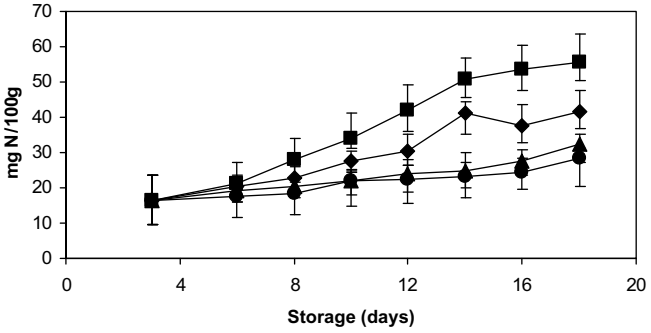

Fig. 3. Changes in total volatile basic nitrogen (TVB-N) of fresh swordfish fillets during storage at 4 °C under aerobic packaging (A; ■), aerobic packaging with thyme oil (0.1% v/w, AT; ♦), modified atmosphere packaging (M; ▲) and modified atmosphere packaging with thyme oil (0.1% v/w, MT; ●). Each point is the mean of three samples taken from two replicate experiments ($n = 3 \times 2 = 6$). Error bars show SD.

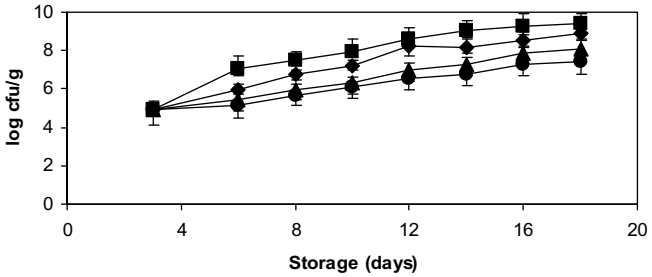

Fig. 4. Changes in total viable counts of fresh swordfish fillets during storage at 4 °C under aerobic packaging (A; ■), aerobic packaging with thyme oil (0.1% v/w, AT; ♦), modified atmosphere packaging (M; ▲) and modified atmosphere packaging with thyme oil (0.1% v/w, MT; ●). Each point is the mean of three samples taken from two replicate experiments ($n = 3 \times 2 = 6$). Error bars show SD.

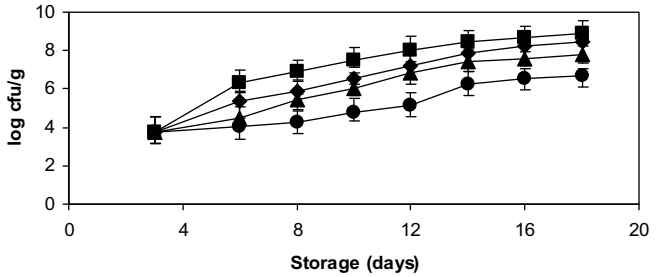

Fig. 6. Changes in H_2S-producing bacteria (including *Shewanella putrefaciens)* of fresh swordfish fillets during storage at 4 °C under aerobic packaging (A; ■), aerobic packaging with thyme oil (0.1% v/w, AT; ♦), modified atmosphere packaging (M; ▲) and modified atmosphere packaging with thyme oil (0.1% v/w, MT; ●). Each point is the mean of three samples taken from two replicate experiments ($n = 3 \times 2 = 6$). Error bars show SD.

Initial populations of pseudomonads (Fig. 5) and H_2S-producing bacteria (Fig. 6) were ca. 4.0 and 3.7 log cfu/g, respectively. Pseudomonads and H_2S-producing bacteria (including *S. putrefaciens)* reached similar final counts of ca. 9.1, 8.4 log cfu/g and 8.9 and 8.4 log cfu/g, respectively, for A and AT swordfish samples. Interestingly, M and MT swordfish samples, after 18 days of storage, had significantly lower ($P < 0.05$) counts (7.4, 6.5 cfu/g for pseudomonads and 7.8, 6.7 log cfu/g for H_2S-producing bacteria) than had the control samples.

Initial counts were ca. 4.0 log cfu/g (LAB) and 2.5 log cfu/g (*Enterobacteriaceae*), the latter indicating a good hygiene of the marine environment from which the swordfish were caught, as well as good fishing practices and subsequent handling. Final populations of LAB (7.7, 7.0, 7.6 and 7.1 log cfu/g) and *Enterobacteriaceae* (7.0, 6.3, 5.8 and 5.3 log cfu/g) were recorded for treatments A, AT, M and MT, respectively, producing lower counts ($P < 0.05$) than those obtained for Pseudomonads and H_2S-producing bacteria (including *S. putrefaciens*).

3.3. Sensory evaluation

The results of the sensory evaluation (odour, taste and overall acceptance) of cooked swordfish fillet samples are presented in Fig. 7a–c. Odour, taste and overall acceptance scores for swordfish samples stored under aerobic conditions, with or without thyme essential oil, under MAP conditions, with or without thyme essential oil, showed a similar pattern of decreasing acceptability (Fig. 7 a–c). As determined by sensory analysis (odour attribute) data, the observed shelf-life of swordfish fillets was longest for MT swordfish samples (15 days), followed by AT, M (12–13 days) and control (A) samples (8 days). Similarly, analysis of sensory (taste) data gave shelf-lives of 16, 12, 9 and 8 days for MT, M, AT and controls (A) swordfish samples, respectively. The results of the shelf-life, as judged by both sensory attributes, for MT, M and A swordfish samples, were in good agreement, with the exception of swordfish AT samples, where a difference of 3–4 days in shelf-life was noted.

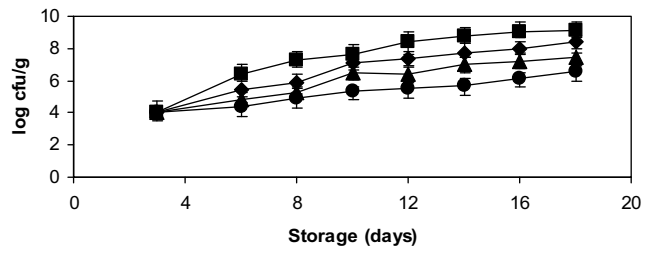

Fig. 5. Changes in *Pseudomonas* spp. of fresh swordfish fillets during storage at 4 °C under aerobic packaging (A; ■), aerobic packaging with thyme oil (0.1% v/w, AT; ♦), modified atmosphere packaging (M; ▲) and modified atmosphere packaging with thyme oil (0.1% v/w, MT; ●). Each point is the mean of three samples taken from two replicate experiments ($n = 3 \times 2 = 6$). Error bars show SD.

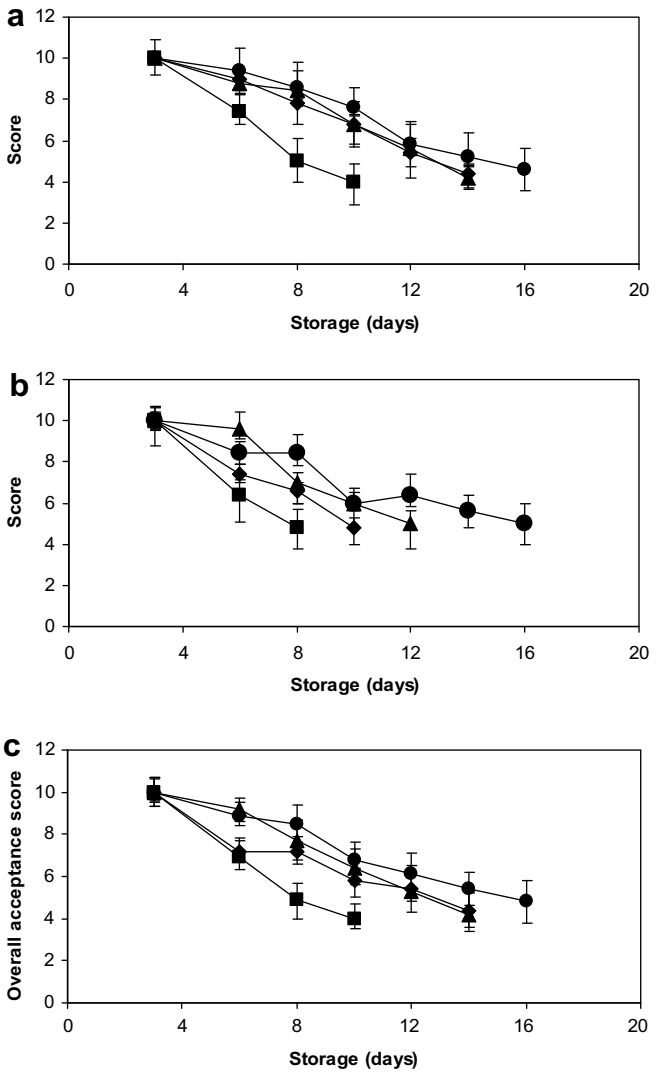

Fig. 7. Changes in odour (a), taste (b) and overall acceptance (c) scores of fresh cooked swordfish fillets during storage at 4 °C under aerobic packaging (A; ■), aerobic packaging with thyme oil (0.1% v/w, AT; ♦), modified atmosphere packaging (M; ▲) and modified atmosphere packaging with thyme oil (0.1% v/w, MT; ●). Each point is the mean of three samples taken from two replicate experiments ($n = 3 \times 2 = 6$). Error bars show SD.

Table 1
Shelf-life of fresh swordfish fillets during storage at 4 °C under aerobic packaging (A), aerobic packaging with thyme oil (0.1% v/w, AT), modified atmosphere packaging (M) and modified atmosphere packaging with thyme oil (0.1% v/w, MT).

Packaging treatment	TMA-N[a,e]	TVB-N[b,e]	Shelf-life (days) Microbiological analysis (TVC)[c,e]	Sensory analysis[d,e]
A	7	7	6	8**
AT	8–9	9	9	13
M	8–9	14–15	12	13
MT	10–11	16–17	15	15–16

[a] Based on a TMA-N upper limit value of 3.72 mgN/100 g fish muscle.
[b] Based on a TVB-N upper limit value of 24.5 mgN/100 g fish muscle.
[c] Based on a microbiological TVC limit value of 7 log cfu/g.
[d] Based on the overall acceptance score.
[e] Data obtained from Figs. 2–4 and 7, respectively.

3.4. Determination of shelf-life of swordfish samples based on physicochemical, microbiological and sensory analyses

Table 1 summarises data obtained from Figs. 2–4 and 7 on the shelf-life of A, AT, M and MT swordfish samples based on physicochemical, microbiological and sensory analyses. Based on TMA-N data (Fig. 2 and an upper limit value of 3.72 mg N/100 g fish muscle), shelf-lives of 7, 8–9 and 10–11 days for A, AT or M and MT swordfish samples were obtained, respectively. Similarly, as determined by TVB-N data (Fig. 3 and an upper limit value of 24.5 mg N/100 g fish muscle) shelf-lives of 7, 9, 14–15 and 16–17 days for A, AT, M and MT swordfish samples were recorded, respectively. Using TVC data (Fig. 4 and based on a TVC value of 7 log cfu/g as the limit of microbiological acceptability) microbiological shelf-lives of 6, 9, 12 and 15 days were obtained for A, AT, M and MT swordfish samples, respectively (Table 1). Finally, sensory analysis of the overall acceptance data (Fig. 7c) gave shelf-lives of 8, 13 and 15–16 days for A, AT or M and MT swordfish samples, respectively. Interestingly, results of the shelf-life of A, M and MT swordfish samples obtained from the physicochemical (TVB-N data), microbiological and sensory analyses are in good correlation, with the exception of AT swordfish samples, where a difference of 4 days is noted. Sensory evaluation and microbiological shelf-life of the aforementioned samples were in good agreement (Table 1).

4. Discussion

It is a common practice in studies of preservation of foods, including fresh fish, to determine the shelf-life of a product, either using sensory or microbiological analyses. Additionally, physicochemical indices, including, pH, TBA, TMA-N and TVB-N, have also been suggested to play a role in monitoring quality and freshness of fresh fish and seafoods (Gram & Dalgaard, 2002). In the present study, the aforementioned physicochemical indices were used in order to determine both quality and shelf-life of swordfish samples, stored aerobically or under modified atmosphere in the absence and presence of thyme essential oil.

With regard to pH values, irrespective of treatment, no specific trend was noted, and these values are in agreement with those values reported in the literature for fresh swordfish fillets stored in retail packages containing various CO_2 atmospheres (Lannelongue et al., 1982; Oberlender et al., 1983). Therefore, the parameter of pH is not useful as a physicochemical index of quality decay or in predicting the shelf-life of swordfish samples.

Lipid oxidation, as determined by malondialdehyde values (MDA), for A and M swordfish samples, was variable, indicative of no specific oxidative rancidity trend. According to Auburg (1993), TBA values do not represent the actual lipid oxidation rate, due to several interactions between MDA and amino acids, proteins, glucose and other fish constituents during storage (Fernandez, Perez-Alvarez, & Fernandez-Lopez, 1997). This observation is in agreement with previous reports (Fernandez et al., 1997; Goulas & Kontominas, 2007; Pournis et al., 2005; Ruiz-Capillas & Moral, 2001).

Lannelongue et al. (1982) and Oberlender et al. (1983) suggested 5 mg N/100 g as a limit of acceptability for swordfish. As previously noted for pH, likewise TBA (as an index of lipid oxidation/rancidity) cannot be used to assess quality decay of swordfish under the conditions of the present study.

With regard to the volatile amines, TMA-N and TVB-N, both have been used in studies of quality and freshness of various fish and seafood (Sivertsvik et al., 2002). Given the great variation in TMA-N acceptability limits for various fish species reported, and TMA-N values shown in Fig. 2, as well as data based on sensory (overall acceptance) scores (Fig. 7c) and microbiological (TVC) data (Fig. 4), a more realistic TMA-N limit of acceptability for Mediterranean swordfish, of ca. 3.72 mg N/100 g, may be proposed. On the basis of this limit, A, AT, M and MT swordfish fillets exceeded this value on days 7, 8–9 and 10–11 days, respectively, which could be used to mark the shelf-life of swordfish fillets (Table 1). Lower production of TMA-N in AT and MT swordfish samples may be due to the antibacterial properties of thyme oil. Interestingly, MT treatment (combination of packaging and essential oil) effectively controlled the population of H_2S-producing bacteria including *S. putrefaciens* (known to play important role in fish kept under MAP conditions) to less than 7 log cfu/g during refrigerated storage of swordfish (Fig. 6), since it is known that cell concentrations of 7 log cfu/g of these bacteria are responsible for increased TMA-N production. Therefore, of all the treatments, MT combination managed to control growth of H_2S-producing bacteria and limit excessive TMA-N formation.

A TVB-N value of 35 mg N/100 g has been proposed as an upper acceptability limit for spoilage initiation for fresh fish, by the European Commision (Commission of the European Community, 1995). With regard to data on the limit of acceptability limit of TVB-N for swordfish, Lannelongue et al. (1982) and Oberlender et al. (1983) proposed a TVB-N value of 25 mg N/100 g whilst, recently, Muratore and Licciardello (2005) proposed a value of 35 mg N/100 g as the onset of spoilage for smoked swordfish slices. On the basis of the present data (TVC, sensory evaluation), a similar TVB-N limit value of 24.5 mg N/100 may be proposed for the initiation of fresh Mediterranean swordfish spoilage. Swordfish samples A, AT and M, MT exceeded the proposed (in our study) upper TVB-N acceptability limit on days 7, 9, 14–15 and 16–17, respectively, values that could be assigned as the physicochemical (using TVB-N as a parameter) shelf-life of swordfish samples under the experimental conditions of the present study (Table 1).

The inhibition of TVB-N production in swordfish samples may be attributed to the effects of MAP (treatments M and MT) and/or with the combined synergistic effect of thyme oil (treatment MT), the latter known to possess antibacterial properties due to it phenolic constituents, carvacrol and thymol (Burt, 2004). In

other studies, Harpaz et al. (2003) found that addition of thyme or oregano oil (0.05% v/v) to sea bass, maintained TVB-N values below levels proposed by the author's acceptability limit of 30 mg N/100 g for 35 days, during storage at 0–2 °C. Mahmoud, Yamazaki, Miyashita, Shin, and Suzuki (2004) reported a TVB-N value of 30 mg N/100 g after 12 days of storage at 5 °C, after dipping carp fillets in a solution of 0.5% carvacrol and thymol (v/v), whereas the control reached this value after only 4 days.

Of these volatile amines, TVB-N may be proposed as a quality decay index of fresh swordfish, under the present conditions of the study, since evaluation of the shelf-life as determined by this parameter, was generally in good agreement with respective values of shelf-life, determined using microbiological (TVC) and sensory (overall acceptance data) (Table 1). To our knowledge, very little information is available in the literature on the effect of EOs on TMA-N and TVB-N production in fish/seafood.

Low initial (day 3) TVC of swordfish fillets, ca. 4.9 log cfu/g, indicates good fish quality, in agreement with results (4.6 log cfu/g) reported by Lannelongue et al. (1982) for fresh swordfish fillets. The combination of MAP and thyme essential oil resulted in a shelf-life extension of 9 days, attributed to the antimicrobial effects of the thyme essential oil and especially to its phenolic components, thymol and carvacrol, known to exert antimicrobial activity (Burt, 2004; Lambert et al., 2001; Holley & Patel, 2005) (Table 1). In other studies, Mahmoud et al. (2004) found that dipping carp fillets in carvacrol/thymol solution (1%) both reduced the initial TVC and extended the shelf-life from 4 days to at least 12 days at 5 °C, according to microbiological results. Harpaz et al. (2003) observed that treatment with 0.05% (v/w) thyme and/or oregano oil increased the shelf-life of Asian sea bass to 33 days at 0–2 °C, as compared to 12 days for the control. Furthermore, Ouattara, Sabato, and Lacroix (2001) found that the shelf-life of shrimp was extended by using a combined treatment of γ-irradiation, thymol oil and *trans*-cinnamaldehyde.

Under MAP conditions of the present study, as expected, and due to the antimicrobial effect of CO_2 mainly on aerobic spoilage bacteria (i.e. pseudomonads), a shelf-life extension of 6 days was recorded, in agreement with other researchers' findings for swordfish fillets stored under 40% CO_2/60% N_2 (Lannelongue et al., 1982).

Of the packaging treatments used in the present study, M (5% O_2/50% CO_2/45% N_2) and MT (5% O_2/50% CO_2/45% N_2 with added 0.1% v/w thyme EO) were the most effective for the inhibition of pseudomonads and H_2S-producing bacteria in swordfish, known to cause spoilage of fresh fish (Gram & Dalgaard, 2002). Both of these species, reaching high numbers in A, AT and M swordfish samples, were dominant and are, therefore, likely to be the specific spoilage organisms of swordfish, although this hypothesis was not investigated further in the present study. Similarly to our findings, *Pseudomonas* spp. dominated the microflora of swordfish steaks stored in air and under CO_2-enriched atmospheres (except for 100% CO_2) (Lannelongue et al., 1982; Oberlender et al., 1983).

LAB and *Enterobacteriaceae* (to a lesser extent), being facultative anaerobic bacterial species, were also found to be part of the natural microbial flora of swordfish fillets, irrespective of packaging treatment (results not shown). Obviously, the use of MAP did not inhibit the growth of LAB, due to their tolerance against the action of CO_2. Likewise, Ordonez, Lopez-Galvez, Fernandez, Hierro, and de la Hoz (2000), Masniyom et al. (2002), Pournis et al. (2005) and Stamatis and Arkoudelos (2007) found high final counts of LAB in the microbial flora of refrigerated fish species, stored under various MAP conditions. Use of thyme oil with either air or MAP (treatments AT, MT) did not have a significant effect ($P > 0.05$) on the reduction of the LAB population. Likewise, Tassou, Drosinos, and Nychas (1995) observed that the addition of olive oil/lemon juice/oregano oil to cold fresh fish fillets, under MAP, reduced the final LAB counts, by only 0.5 log cfu/g, compared to the control. The limited action of thyme oil is attributed to the high tolerance of LAB toward the action of essential oils, due to their ability to generate ATP and to deal with conditions of osmotic stress (Holley & Patel, 2005). With regard to the *Enterobacteriaceae*, treatments M and MT produced lower counts ($P < 0.05$) throughout the entire storage period than did the control samples, probably due to the CO_2 action and the antimicrobial effect of the oregano oil. Our results for *Enterobacteriacea* are in agreement with findings of fresh fish species stored under MAP conditions (Ordonez et al., 2000; Pournis et al., 2005; Stamatis & Arkoudelos, 2007).

It is noteworthy, that the presence of thyme oil (0.1% v/w) in cooked AT and MT swordfish samples produced a distinct but sensorially acceptable pleasant odour, well received by the panellists. This could partly explain the discrepancy arising in the difference of the shelf-life of AT samples (4 days), as determined by both microbiological and sensory analyses. The use of thyme oil at higher concentrations (>0.1%) would probably result in a further increase of shelf-life of swordfish, but such EO concentrations would probably impart unpleasant sensorial effects (bitter taste and strong odour) on the quality of swordfish fillets.

Of all the treatments investigated in the present study, MT treatment was the most effective in terms of shelf-life extension of swordfish slices (Table 1), as it gave the lowest final TVC count (<8 log cfu/g), resulted in an organoleptically acceptable product (in terms of both odour and taste attributes and as judged by the overall sensory acceptance score), finally leading to minimal formation of chemical decay markers (both TMA-N and TVB-N), compared to the control samples.

It must be noted that our study is the first one reporting use of thyme essential oil and packaging on the shelf-life evaluation of Mediterranean swordfish (fillets) during storage at 4 °C, using physicochemical, bacteriological and sensory analyses. Further studies are needed with regard to preservation of fresh swordfish using natural antimicrobials, including essential oils, in view of increasing consumer demand for (preservative-free) fresh fish and sea foods, and more specifically the need arises so as to establish optimum essential oil concentrations that will maximise shelf-life, whilst at the same time maintaining desirable freshness and quality (sensorial) characteristics.

5. Conclusions

Based primarily on sensory data, the shelf-lives of fresh refrigerated Mediterranean swordfish were 8 and 13 days under aerobic and MAP conditions, respectively. Addition of 0.1% thyme essential oil extended the product's shelf-life under aerobic conditions by 5 days, whereas the combination of MAP and thyme oil resulted in a significant shelf-life extension of the swordfish fillets, i.e. by approximately 7½ days, according to sensory data, as compared to the control sample.

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