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Shelf-Life of Smoked Eel Fillets Treated with Chitosan or Thyme Oil

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Abstract

The present study examined the effect of natural antimicrobials: Chitosan, thyme oil and their combination, on the shelf-life of smoked eel fillets stored under vacuum packaging (VP) at 4°C. Based on sensory odor data smoked eel fillets had a shelf-life of 35 (control), 42 (thyme treated and >49 (thyme, chitosan-thyme treated) days. The thiobarbituric acid value (TBA) value of the control eel sample was significantly higher than the chitosan-thyme-treated eel samples. The use of chitosan singly, or in combination with thyme oil reduced lipid oxidation (TBA) of the smoked eel samples. A trimethylamine nitrogen (TMA-N) value of 10 mg N/100 g, could be suggested as an indication of smoked eel spoilage initiation. Control and treated eel reached total volatile basic nitrogen (TVB-N) values of 13.1-31.5 mg N/100 g below the maximum permissible level of TVB-N in fish and fishery products. Eel samples reached the value of 7.0 log cfu/g (Total Plate Count,TPC) on days 35 (smoked) and 42 (thyme treated), whereas both chitosan and chitosan-thyme treated eel samples never reached this limit value. Results of our study show thyme or chitosan (singly, or in combination) inhibit the growth of mesophilic bacteria and extend the shelf-life of smoked eel.

1 Introduction

European eel is a commercially important species because of its white flesh, flavour and high fat content. Currently the species, mostly farmed is known as the European eel (*Anguilla Anguilla*) growing in fish farms, transported usually alive in tanks to the retail market. Consumers may buy eels either as a freshly available processed (usually after gutting and beheading processes) product in the market, or buy it in fillets that have been processed and vacuum packaged. Eels are known to have a high fat content, being rich in ω3 lipids, and in the last decade acquacultured fish or seafood consumption has been increasing, therefore, the potential of this under-utilized species has been growing and eels have been attaining a gourmet status throughout the world.

As with all seafood and freshly caught fish, freshness and initial microbial load may affect the shelf-life of these products and so far various methods such ice storage [1], modified atmosphere packaging (MAP) [2] and recently use of herbal extracts (myrtle, laurel) have been suggested [3]. Although the freshness, quality parameters, relating to the shelf-life of Mediterranean fish, either those freshly caught or cultured from open sea, have been extensively studied, there is limited information available on eel freshness and quality characteristics, including smoked eel. In one of these studies, Ozogul et al. [1] reported that the shelf-life of iced eel and eel without ice was 12–14 days and 5–7 days, respectively. In another study, Arkoudelos et al. [2] reported that all raw eel samples received acceptable sensory scores during the first 11±1 days of storage in atmospheric air, 11±1 days of storage in vacuum and finally 18±1 days of storage in MAP conditions. Using the microbial quality indicators the shelf life of eel packaged in air, vacuum and MAP was estimated to be more than 18, 28 and 34 days, respectively. Recently, Ozogul et al. [3] investigated the effects of

myrtle and laurel extracts on the sensory, chemical and microbiological quality of European eel (*Anguilla anguilla*). In this study, based primarily on sensory assessment, vacuum- packaged European eel reached the limits of acceptance 12 days for the control, 16 days for laurel and 20 days for myrtle. Therefore, the aim of the present study was to investigate the effects of the aforementioned antimicrobials on the shelf-life of smoked, vacuum-packaged and refrigerated European eel (*Anguilla anguilla*).

2 Materials and Methods

2.1 Eel samples

Fresh smoked eel (skinless) fillets, ca. 200 g or 15 cm (L) x 3 cm (W) each, Nitsiakos, S.A., Ioannina, Greece) were provided by a local acquaculture seafood processing company (Geitonas, E., Co., Psathtopi, Arta), within 12 h of slaughter in insulated polystyrene boxes on ice flakes. Smoking of eel fillets was accomplished according to the seafood company's processing method. Eel samples were subsequently kept under refrigeration in a cooling incubator (2^{o}C) before the addition of the antimicrobials).

2.2 Preparation of chitosan and thyme EO solutions

Chitosan of low molecular weight (MW; 340) in powder form from crab shells was purchased from Aldrich Company (Athens, Greece). Moisture content was less than 10% and chitosan had a deacetylation degree of 75-85%. A stock solution of chitosan was prepared by dissolving 2.0 g in 100 ml of 2% (w/v) glacial acetic acid and stirred overnight at room temperature (final chitosan concentration = 2.0% w/v). Pure thyme EO (Kokkinakis S.A., Athens, Greece) was used. All media, solvents and chemicals of analytical grade (Analar) were obtained from Aldrich Company (Athens, Greece). Distilled water was used to prepare all media and solutions.

2.3 Application of the antimicrobials to the smoked eel samples

The antimicrobials were added to the smoked eel fillets, either singly, or sequentially using the following procedure: An eel fillet (ca. 200 g ± 10 g) was transferred aseptically into an open sterile packaging pouch, containing 100 ml of chitosan solution (2.0% w/v). Each fillet was individually dipped and remained in contact with the chitosan solution for 1.5 min. Immediately after dipping, the excess solution was drained off on a rack that was previously sterilized (absolute alcohol) and this procedure was done under aseptic conditions in a sterile cabinet. Each sample was then vacuum packaged into a clean plastic pouch. Thyme essential oil (EO) was added onto the eel samples (0.3 ml of oil onto 100 g) undiluted at a final concentration of 0.3% v/w using a Volar micropipette, also packaged as previously. Finally, for the combined antimicrobial treatment, chitosan solution was applied first to the eel samples, followed by the thyme EO, both added at concentrations, previously applied**.** It must be noted that the selection of the final optimum concentration of each antimicrobial, applied to the eel fillets, was established following preliminary microbiological analysis (determination of the mesophilic total plate count (TPC) and sensory evaluation of the eel samples, treated with the aforementioned antimicrobials at selected concentrations in the range of 1-2 % w/v (chitosan) and 0.1-1.0% v/w (thyme EO) (results not shown). Additionally, to the treatments of the present study, preliminary experiments tested the effect of acetic acid on the quality of eel fillets. Samples were analysed sensorially (cooked) and microbiologically (TPC) (results not shown). It was concluded that addition of acetic acid to the eel fillets did not negatively affect the sensory parameters of the smoked eel fillets.

2.4 Packaging of samples

A fresh skinless smoked eel fillet (200 g ± 10 g) was transferred aseptically into an open low density polyethylene/polyamide/low density polyethylene pouch (VER PACK, Thessaloniki, Greece), 75 μm in thickness having an oxygen permeability of 52.2 $cm^3/m^2/day/atm$, at 75% relative humidity (RH), 23°C, a carbon dioxide permeability of 191 $cm^3/m^2/day/atm$ at 0% RH, 23°C and a water vapour permeability of 2.4 $g/m^2/day$ at 100% RH, 23°C). The eel samples were subdivided into 4 lots: S (control, smoked, no antimicrobials added), ST with added thyme EO at 0.3% v/w; SC with added chitosan at 2.0% w/v and SCT with added chitosan 2.0% v/w and thyme EO 0.3% v/w. Pouches were heat-sealed using a BOSS model N48 packaging machine (Bad Homburg, Germany) after air was drawn (vacuum storage). Eel samples (control and treated) were kept under VP conditions and refrigeration (4^oC) for a period of 35 (S), 42 (ST) and (SC, SCT) 49 days.

2.5 Sensory analysis

Each eel sample (skinless smoked fillet, ca. 200 g) was cooked in a microwave oven at high power (700 W) for 10 min. A panel of seven judges experienced (laboratory-trained, mostly staff and postgraduate students) in eel evaluation was used for sensory evaluation. All panelists who evaluated the sensory attributes of cooked eel had previously participated in training sessions to become familiar with the sensory characteristics of cooked eel fillets**.** Panelists were asked to evaluate taste and odor of the cooked samples. Acceptability as a composite of odor, and taste was estimated using a scale ranging from 0-9. In this scale, scores between 7.0 and 9.0 indicated, “extremely liked,” scores between 4.0 and 6.9 indicated “liked,” and 3.9 was the limit of acceptability [4].

2.6 Chemical analysis

Thiobarbituric acid (TBA), Trimethylamine nitrogen (TMA-N), Total volatile basic nitrogen (TVB-N) were determined according to the methods proposed by Pearson [5], AOAC [6], Malle and Poumeyrol [7], respectively.

2.7 Microbiological analysis

Each smoked eel fillet (25 g) was mixed with 225 ml of 0.1% sterile peptone water (Merck, Darmstadt, Germany) in a sterile filtered stomacher bag (Seward Ltd., London, UK). The mixture was stomached for 1 min. For microbial enumeration, 0.1 ml samples of serial dilutions (1:10, diluent, 0.1% peptone water) of turkey homogenates were spread on the surface of agar plates. Mesophilic bacteria were determined using Plate Count Agar (PCA, Merck, Darmstadt, Germany), after incubation for 2 days at 37^{o}C. *Pseudomonas* spp. were enumerated on cetrimide fusidin cephaloridine agar (CFC, Oxoid code CM 559, supplemented with SR 103, Oxoid, Basingstoke, UK) and incubated at 25^{o}C for 2 days. *Shewanella* spp. was determined on Iron agar medium (only black colonies) and incubated at 30^{o}C for 2 days. Finally, yeasts were enumerated on Rose Bengal Chloramphenicol (RBC) selective agar (Merck, Germany) plates using the surface spreading technique and plates were incubated at 25ºC for 3-5 days in the dark.

All procedures were performed in compliance with relevant laws and institutional guidelines. The appropriate institutional committee(s) have approved them.

2.8 Statistical analysis

Experiments were replicated twice (n=2) on different occasions with different eel fillet samples. Analyses were run in triplicate for each replicate. Results are reported as mean values ± standard deviation (S.D.). Data were subjected to analysis of variance (ANOVA). The least significant difference (LSD) procedure was used to test

for differences between means ($P < 0.05$). Microbiological counts were converted to log cfu/g and were subjected to analysis of variance (ANOVA) using the software Stat graphics (Statistical Graphics Corp., Rockville, MD, USA).

3 Results and discussion

3.1 Sensory changes of vacuum packaged smoked eel fillets in the absence and presence of antimicrobials

The results of the sensory evaluation (odor, taste attributes) of the untreated (S) and treated (ST, SC, SCT) eel samples stored under vacuum at 4^{o}C are presented (Fig. 1a, 1b). Individual odor (Fig. 1a), taste (Fig. 1b) and overall acceptability (not shown) scores showed a similar pattern of decreasing acceptability for the control and treated eel samples, in agreement with results reported by Ozogul et al. [3] and Kucukgulmez et al. [8] on vacuum packaged eel. Of the treatment groups examined in our study, SC retained the highest odor and taste scores, especially after day 14 and up to final day 49 of storage, followed by the SCT eel samples. The presence of thyme EO in the latter resulted in a slightly bitter-like odour and metallic taste, thus accounting for the higher preference of panellists for the chitosan treated smoked eel (SC, SCT) samples. The presence of chitosan in the SCT eel samples alleviated the bitterness of thyme EO, imparted a "lemony" odor and taste that was well received by the panellists. Similarly, SC eel fillets also retained a marked, distinct and pleasant state of freshness, similar to that noted for the SCT eel. Tsiligianni et al. [9] also reported a "lemony" taste on cooked swordfish fillets treated with chitosan. In other studies, Similarly, Tsai et al. [10] observed that the shelf-life of salmon fillets dipped in chitosan solution (1% in 0.1 N HCl) was extended from 5 to 9 days, whereas the application of myrtle and laurel extracts to eel fillets led to an improvement in the odour and taste of the samples [3]. In this study, panellists preferred eel samples that

were treated with myrtle extract [3]. Recently Kucukgulmez et al. [8] reported that both extracted chitosan (from *Metapenaeus stebbingi* shells) and commercially purchased chitosan, added at 0.1 and 1% (w/v) concentrations to vacuum-packaged eel fillets extended the shelf-life of eel fillets by 6 days compared with the control group, whereas MAP storage (CO_2/40%; N_2/30%; O_2/30%) in combination with refrigeration (0^{o}C), extended the shelf-life of eels to 18 days, thus a 7-day extension, compared to the shelf-life of 11 days, under air packaging conditions [2].

Based on sensory odor data (primarily, Fig. 1a) and considering a score of 3.9 (as marking the end of sensorial shelf-life) smoked eel fillets had a shelf-life of 35 (S), 42 (ST) and >49 (SC, SCT) days. Our results, based on sensory odor and taste scores revealed a preference of panelists for the chitosan treated (SC, SCT) eel samples, compared to the thyme treated eel fillets. It must be noted that the addition of an effective concentration of an EO, i.e. thyme EO, in terms of antimicrobial action, must be carefully monitored in view also of a possible negative effect (bitterness) of the EO on the organoleptic attributes on the eels, if applied at high concentration. Present results, in combination with current, limited knowledge on the potential use of natural antimicrobials, i.e. chitosan and plant EOs as "natural" preservatives in foods, leads to the general conclusion that their use (singly, or in combination) could expand its application for shelf-life extension of seafood.

3.2. Chemical changes of vacuum packaged smoked eel fillets in the absence and presence of antimicrobials

Of the chemical indices monitored, TBA, TMA-N and TVB-N mostly have been used to assess fish/seafood freshness and quality [11]. In our study changes in TBA, TMA-N and TVB-N values were monitored in control (S) and treated (ST, SC and SCT) eel fillet samples (Figs. 2a, 2b, 2c).

TBA value is a major chemical index to measure the degree of oxidative rancidity, and has been used in numerous studies, including seafood, of high fat lipid content [11]. Changes in TBA values in all groups are given in Fig 2a. Initial TBA value of eel fillets was 2.0 mg MDA/kg, increasing to 4.2 mg MDA/kg on day 35, whereas a lower initial value (0.4 mg MDA/kg) was reported by Ozogul et al. [3] for eel fillets stored under vacuum. On day 35 the TBA value of the control (S) eel sample ($P < 0.05$) was significantly higher than the treated (SC, SCT) samples (Fig. 2a). Significant differences were reported for the TBA values of myrtle extract treated samples as compared to the control group [3]. In our study the lower TBA values in the treated SC, SCT eel samples could be due to the high antioxidant activity of chitosan and thyme EO, reducing the level of oxidation in the eel samples. In another study, dipping of carp fillets in a carvacrol/thymol solution (1% v/v) resulted in TBA values of < 0.5 mg MDA/kg during a 20-day storage period, whereas at the same time in the untreated carp fillet a TBA value of 1.4 mg MDA/kg, was noted [12].

Low initial TMA-N value of 0.15 mg N/100 g, is indicative of eel fillet freshness (Fig. 2b) increased to reach a value of of 10.5 mg N/100 g in the control (S) eel

sample on final day 35. An increasing trend in TMA-N values was noted for the ST eel samples, whereas a lag phase (up to day 21) was noted in TMA-N formation for the SC and SCT eel samples, resulting in significantly lower ($P < 0.05$) values of 2.25 and 1.5 mg N/100 g on final day 49 of storage (Fig. 2b). The use of chitosan singly, or in combination with thyme EO led to a drastic reduction in the TMA-N formation in the eel fillets, in agreement with the findings of Mohan et al. [13] who reported a similar effect on sardine fillets stored on ice. A TMA-N value of 10 mg N/100 has been used as a limit value of fish freshness [11] and to our knowledge no value for TMA-N has been suggested as a threshold limit value of fish freshness. Thus based on our TMA-N data (S, control eel sample, day 35) a TMA-N value of 10 mg N/100 g, could be suggested as an indication of smoked eel spoilage initiation, and such value in our study corresponded to the microbiological TPC limit value of 7 log cfu/g (Fig. 3a). It must be noted that to date limited data are however available on TMA-N formation, and additionally on the effects of thyme EO or chitosan in fresh eels.

TVB-N has been associated with seafood spoilage [11] and a maximum permissible level of TVB-N in fish and fishery products is 35 mg N/ 100 g ([14]. Figure 2c, shows the changes in TVB-N content of vacuum-packaged eel fillets, both in the absence and presence of chitosan and thyme EO. A low initial TVB-N value (day-0) of 2.02 mg N/100 g in eels indicates excellent freshness, whereas a higher value of 7 mg N/100 g was noted for eel stored on ice by Ozogul et al. [1]. TVB-N content of all treatments increased with storage time, and as previously noted, a lag phase of 21 days was noted in the TVB-N production for the ST, SC and SCT eel samples, resulting in significantly lower ($P < 0.05$) values on day 35 (Fig. 2c). Control eel (S) and treated (ST, SC, SCT) eel samples reached TVB-N values of 31.5 mg N/100 g and 18.1, 14.9 and 13.1 mg N/100 g, respectively, on final days 35 and 42, 49 of

storage, and these values are below the maximum permissible level (35 mg N/ 100 g) of TVB-N in fish and fishery products [14]. Ozogul et al. [3] reported TVB-N values of 27.36 mg for vacuum-packaged (untreated) eel fillets, 20.92 mg and 29.27 mg, for laurel and myrtle extract-treated eel fillets on days 12, 16 and 20 of storage, in which all samples in vacuum packages were rejected by the sensory panelists.

3.3. Microbiological changes of vacuum packaged smoked eel fillets in the absence and presence of antimicrobials

Changes of TPC, *Pseudomonas* spp., *Shewanella* spp. and yeasts in S, ST, SC and SCT eel samples are shown (Fig. 3a, 3b, 3c, 3d). The initial (day 0) mesophilic TPC (Fig. 3a) of eel (control S) was ca. 2.85 log cfu/g, in agreement to the findings of Arkoudelos et al. [2] reporting a value of 2.8 log cfu/g for fresh eel. Ozogul et al. [1, 3] reported slightly higher TPC values (3-4 logs) for fresh and iced eel fillets. The initial TPC load (2.85 logs) in the S eel sample may reflect an excellent initial eel quality, even though beheading and gutting processes could have added to the initial TPC load. However the smoking-processing step may have also reduced the initial TPC load of the eel fillets, as it is known, that smoking processes may involve the formation of phenolic compounds that are known to exert an antimicrobial action.

ANOVA showed a significant effect of the antimicrobial treatments (SC, SCT) and storage time on TPC ($P < 0.05$) (Fig. 3a). Eel samples reached the value of 7.0 log cfu/g for TPC, which was considered as the upper acceptability limit for fresh fish/seafood [15] on days 35 (S) and 42 (ST) while SC and SCT eel samples never reached this limit value after a storage period of 49 days. Compared to the control samples (S), a microbiological shelf-life extension of 7 and >14 days was achieved for ST and SC, SCT samples. The 7 and >14 days shelf-life extension for ST and SC,

SCT smoked eel samples could be due to the antimicrobial action of thyme oil's components (especially thymol) and of chitosan, acting on spoilage microorganisms [16]. In a related study, a 5-day microbiological shelf-life extension was obtained for a poultry product (ready to cook chicken-pepper kebab) treated with either thyme oil (0.2% v/w) or chitosan (1.5% w/v) [17]. In the present study and of the treatments examined, SC and SCT treatments were the most effective in inhibiting the growth of mesophilic bacteria (Fig. 3a).

Initial *Pseudomonas* spp. population of 2.2 log cfu/g in eel fillets, reached populations of 6.9 and 6.5 log cfu/g, in the S and ST eel fillets on final days 35 and 42 of storage, respectively, whereas significantly ($P < 0.05$) lower populations were recorded for the treated SCT and SC eel samples during the entire storage period (Fig. 3b). Arkoudelos et al. [2] reported a log value of 7.3 for pseudomonads after 18 days in eel fillets stored in air. Our results show that SC and SCT treatments were the most effective for the inhibition of pseudomonads in eel fillets, probably due to the combined antimicrobial action of chitosan and thyme EO, in agreement with results reported for a poultry product treated with chitosan and thyme oil [17]. It was also reported that the use of thyme and laurel EO treatments reduced the growth of psychrotrophic bacteria in bluefish [18].

In our experiment, *Shewanella* spp., being a facultative anaerobic bacterial group, demonstrated final populations, similar to the *Pseudomonas* spp. counts previously reported, of 6 logs in the S and ST eel fillets eel fillets on final days 35 and 42, respectively, (Fig. 3c). SC and SCT treatments resulted in significantly lower populations ($P < 0.05$) of this species during the entire period of storage. Arkoudelos et al. [2] similarly reported significantly lower ($P < 0.05$) populations in eel fillets stored under VP and MAP conditions. In another study, grape fruit seed extract was

the most efficient against *Shewanella* spp., (including *Shewanella putrefaciens*) in a fresh fish burger [19]. In our study, and of all the treatments examined, it is apparent that chitosan either singly, or with thyme EO, were the most effective treatments agent against *Shewanella* spp. in eel fillets, in agreement with results reported by Tsiligianni et al. [9] for fresh swordfish steaks stored in air.

Finally, with regard to yeasts, all of the antimicrobial treatments (ST, SC and SCT) produced significantly lower ($P < 0.05$) counts in the smoked eel samples on day 35 (Fig. 3d). Chitosan applied, either singly (SC), or in combination with thyme EO oil (SCT) suppressed the growth of these species, and interestingly counts of 2.0-3.0 logs were obtained at the end of storage period (49 days), in agreement with the finding of Arkoudelos et al. [2] demonstrating that vacuum and MAP storage slowed down the growth of yeasts by approximately by 3.0 and 4.0 logs, respectively, in farmed eel stored at 0^{o}C.

4 Conclusions

Based on sensory odor data smoked eel fillets had a shelf-life of 35 (control, smoked), 42 (thyme treated, smoked) and >49 (thyme, chitosan thyme treated, smoked) days. Our results, based on sensory odor and taste scores revealed a preference of panelists for the chitosan treated eel fillets. The use of chitosan singly, or in combination with thyme oil reduced lipid oxidation of the smoked eel samples. A TMA-N value of 10 mg N/100 g, could be suggested as an indication of smoked eel spoilage initiation. Control and treated eel samples reached TVB-N values of 13.1-31.5 mg N/100 g and these values are below the maximum permissible level of TVB-N in fish and fishery products. Results of our study show that the use of thyme or chitosan (singly, or in combination) inhibits the growth of mesophilic bacteria and extend the shelf-life of smoked eel. It must be noted that the addition of an effective concentration of an EO,

i.e. thyme EO, in terms of antimicrobial action, must be carefully monitored in view also of a possible negative effect (bitterness) of the EO on the organoleptic attributes on the eels, if applied at high concentration. Further work is needed on the potential use of chitosan and thyme, or of other plant EOs as "natural" preservatives in foods, including seafood, and more specifically in establishing optimum concentrations of EOs, that could have an antimicrobial action without negatively affecting the products' sensorial characteristics. Further research on quality and shelf-life of seafoods containing or coated with chitosan should be conducted on a scale-up trial under commercial conditions. This would provide us more realistic and practical information needed for actual commercialization of food products containing or coated with chitosans.

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Figure legends

Figure 1

Changes in sensory scores of odor (a) and taste (b) in fresh smoked eel fillets stored under VP (S; ■), stored under VP, treated with thyme EO oil 0.3% v/w (ST; ▲), stored under VP, treated with chitosan 2.0 w/v (SC; ♦), stored under VP, treated with chitosan 2.0 % w/v and thyme EO 0.3% v/w (SCT; ●). Each point is the mean of three samples taken from two replicate experiments (n = 3 x 2 = 6). Error bars show SD.

Figure 2

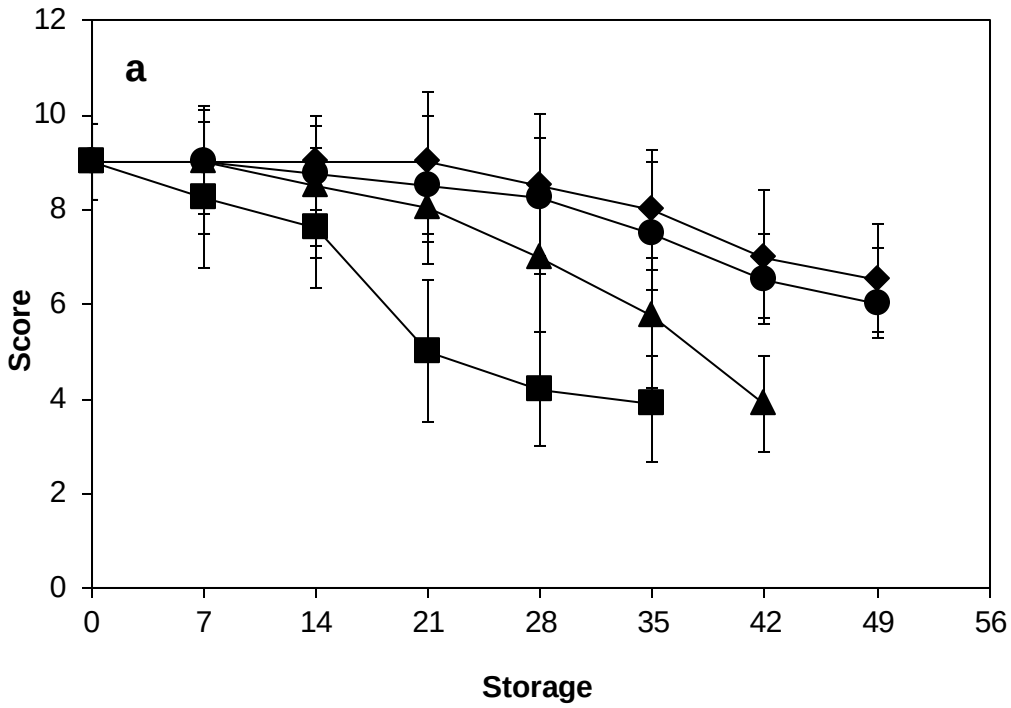
Changes of TBA (a) TMA-N (b), TVB-N (c) values in smoked eel fillets stored under VP (S; ■), stored under VP, treated with thyme EO oil 0.3% v/w (ST; ▲), stored under VP, treated with chitosan 2.0 w/v (SC; ♦), stored under VP, treated with chitosan 2.0 % w/v and thyme EO 0.3% v/w (SCT; ●). Each point is the mean of three samples taken from two replicate experiments (n = 3 x 2 = 6). Error bars show SD.

Figure 3

Changes (log cfu/g) of Total Plate Counts (a), *Pseudomonas* spp (b) and *Shewaella* spp. (c) and yeasts/moulds (d) in smoked eel fillets stored under VP (S; ■), stored under VP, treated with thyme EO oil 0.3% v/w (ST; ▲), stored under VP, treated with chitosan 2.0 w/v (SC; ♦), stored under VP, treated with chitosan 2.0 % w/v and thyme EO 0.3% v/w (SCT; ●). Each point is the mean of three samples taken from two replicate experiments (n = 3 x 2 = 6). Error bars show SD.

FIGURES

Figure 1



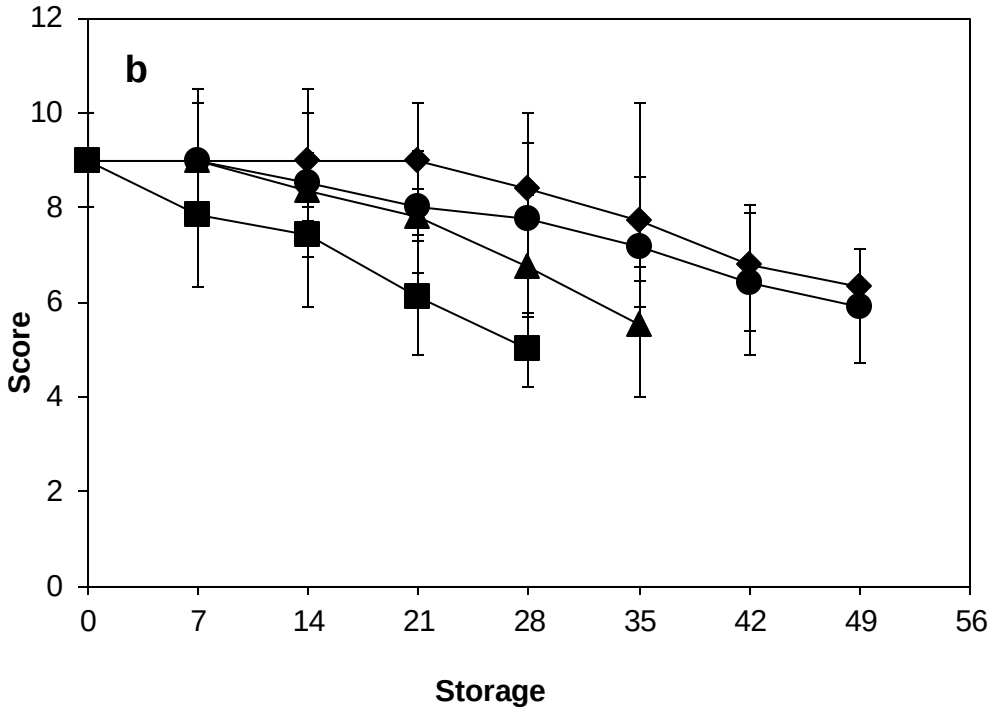
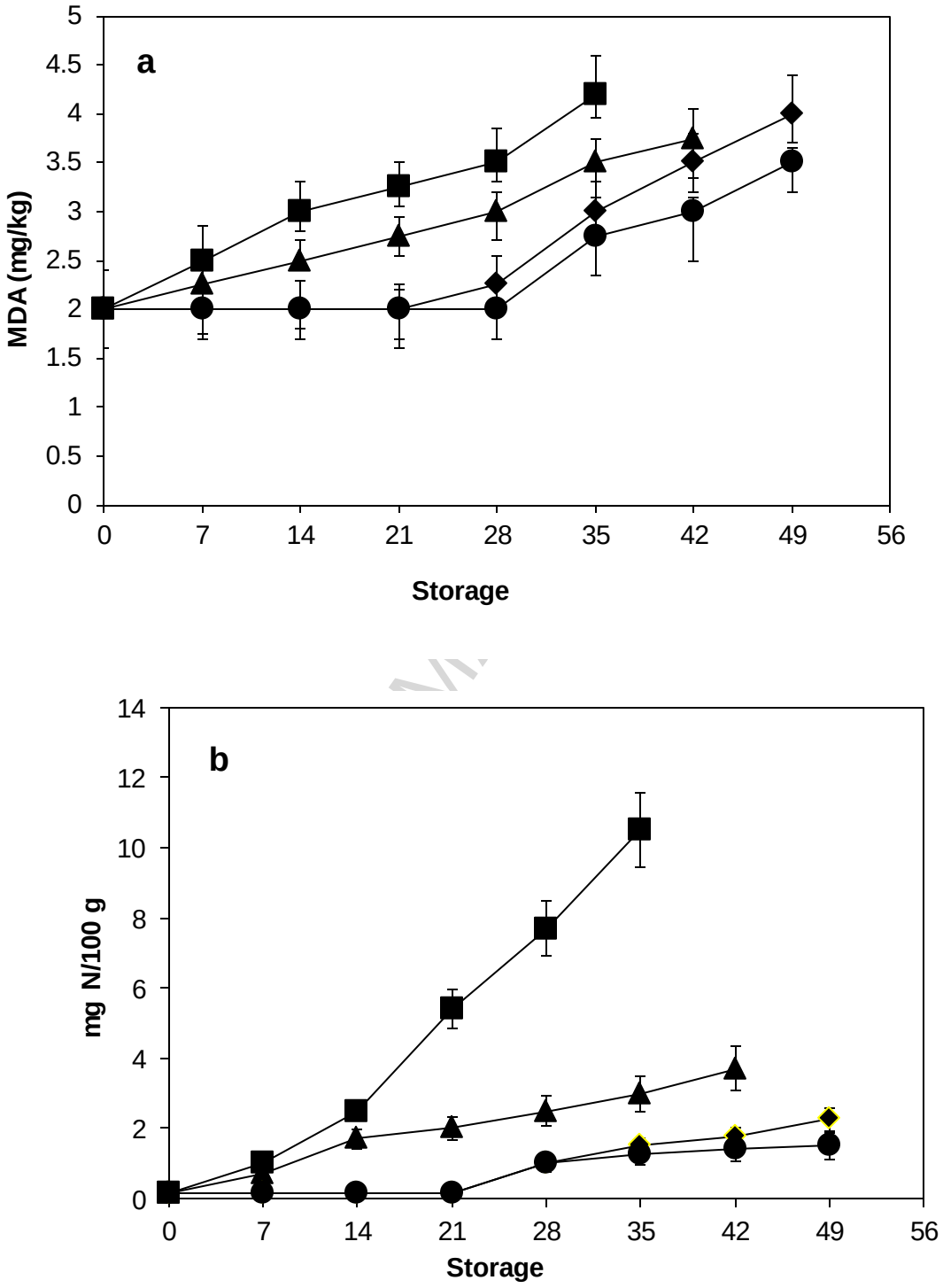


Figure 2



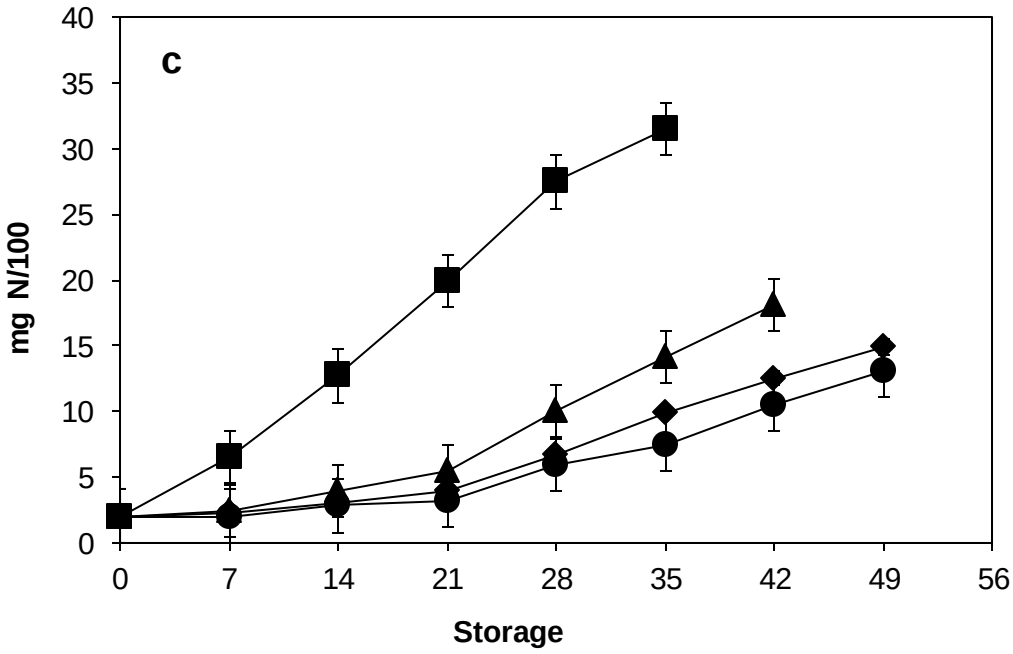


Figure 3