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ORIGINAL PAPER

The Effect of Thyme and Garlic Oil on the Preservation of Vacuum-Packaged Hot Smoked Rainbow Trout (*Oncorhynchus mykiss*)

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Abstract Quality and shelf life of filleted hot smoked rainbow trout packaged in vacuum packaging (untreated) and with thyme oil (TO), and garlic oil (GO)-treated, after vacuum-packaged (VP) conditions stored at 2 °C, were investigated by measurement of microbiological, sensory and physicochemical analyses. Acceptability scores for appearance, odour, taste and texture of untreated and treated smoked trout decreased with storage time. The limit of sensory acceptance was reached after 5 weeks for the untreated samples, after 7 weeks for with thyme oil-treated samples (TO+VP) and after 6 weeks for with garlic oil-treated (GO+VP) samples. Total viable count evaluated that thyme oil- and garlic oil-treated vacuum-packaged samples had good results up to 7 weeks compared with 5 weeks in control vacuum packs. Psychrotrophic counts for untreated and treated samples exceeded 6 log cfu/g after 5 and 6 weeks of cold storage, respectively. Treated samples presented the lowest lactic acid bacteria counts. Total volatile basic nitrogen and trimethylamine nitrogen values gave acceptable results for up to 5 weeks for the untreated samples, 7 weeks for thyme- and garlic oil-treated samples. Treated with thyme oil samples presented the lowest thiobarbituric acid index values. The results obtained from this study showed that the shelf life of hot smoked rainbow trout stored in cold storage (2 °C), as determined by overall acceptability of all data, is 5 weeks for vacuum-packaged samples and 7 weeks for thyme- and garlic oil-treated vacuum-packaged smoked fish.

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Introduction

Smoked fish is a highly nutritious food containing highly unsaturated fatty acids, fat soluble vitamins, essential minerals as well as proteins containing the amino acids essentials for human beings (Bilgin et al. 2008). Smoked fish has characteristic flavour and colour. Hot smoked fish products made from white fish generally keep better than those made from fatty fish, although shelf life will vary considerably, depending on the amounts of salt and smoke present, the degree of drying and the storage temperature (Bannermann 2001). Generally, hot smoked fish stored in anaerobic conditions (vacuum-packaged) at chilled temperature is very sensitive to deterioration and, based on sensory evaluation, has a limited shelf life ranging from 3 to 4 weeks at refrigerator temperature (Rehbronn and Rutkowski 1982). Lowering of quality is manifested through changes in appearance, odour, colour or texture (Tülsner 1994; Kolsarıcı and Özkaya 1998; Cakli et al. 2006). Hot smoked fish, especially hot smoked rainbow trout, is of considerable economic importance worldwide, particularly in Europe. Improvements in the shelf life of hot smoked fish product can have an important economic feedback by reducing losses attributed to spoilage and by allowing the products to reach distant and new markets.

Spices, seeds, and their oils or extract application has proven to be an effective preservation method for the extension of shelf life of fresh fish (Harpaz et al. 2003; Quitral et al. 2009; Kykkidou et al. 2009). Thyme, oregano, sage, black seed, grape seed, garlic, rosemary oils have natural antimicrobial and antioxidant properties with the

potential to extend the shelf life of foods (Burt 2004; Erkan and Bilen 2010; Erkan et al. 2010).

The effect of thyme and garlic oil (1%: *v*/*w*) in cold storage on vacuum-packaged hot smoked rainbow trout fillets' microbiological status, sensory and chemical characteristics was studied using analysis.

Materials and Methods

Raw Material

Essential oils were purchased from the Ecem Natural Company (Istanbul, Turkey). Undiluted 100% oil of thyme and garlic oil was used. Hot smoked fish was prepared from rainbow trout (*Oncorhynchus mykiss*). Rainbow trout (212.5±2.12 g and 26±1.2 cm) were obtained from a freshwater fish farm of Bağcı in Turkey, in Mai 2008. The fresh fish samples were packed in polystyrene boxes with crushed ice and then transferred to the laboratory. The fish were harvested, beheaded, gutted manually and then washed. The samples (33 gutted fishes) were immersed in brine at a ratio of 1:1 (*w*/*w*) for 24 h at 2 °C. The brine contained 10% NaCl. After brining, the samples were briefly dipped in chilled tap water for 1 h.

Smoking Process

Smoke was produced from oak sawdust with combustion. The processing time in the kiln was divided into two stages: (1) a preliminary drying and cooking period (45 min) at 75 °C; (2) a smoking and partial cooking period at 80 °C (60 min). After cooling at room temperature (60 min), the smoked fish were filleted and divided into three lots.

Essential Oil Treatment, Packaging and Storage of Hot Smoked Rainbow Trout

The following lots of samples were prepared: The first lot of samples comprised the controls (samples in the control treatment had no essential oils added). Thyme oil (TO; second lot) and garlic oil (GO; third lot) were added onto the surface (two sides) of each fillet using a micropipette so as to achieve a final 1% (*v*/*w*) concentration. The volume of thyme and garlic oil was chosen based on information in the literature regarding storage of bluefish and chub mackerel (Erkan and Bilen 2010; Erkan et al. 2010). The control (VP) and oil-treated (TO+VP and GO+VP) hot smoked rainbow trout (three filets for each group of each analysis week) were placed in high-barrier plastic film bags and packaged under vacuum-packaged. Samples were stored in a refrigerator (2±1 °C) and analysed once a week during 7 weeks to determine the shelf life.

Packaging Material and Apparatus

Plastic film bags were obtained from UPM-Kymmene, Walki-Pack, Plastic Films Factory (Valkeakoski, Finland). The characteristics of the plastic film bags were as follows: transmission rate (mL/m^2): O_2, 6.89; CO_2, 5.42; N_2, 2.48, at 4 °C; vapour permeability, 7.86 mL/m^2 at 37.8±1 °C, 90± 2% RHg/m^2 days atm. Samples were packed in a Komet vacuum-packaging machine PAXX model (Germany).

Analyses

Microbiological Analyses

Sample Preparation Smoked fish (25 g) obtained from each smoked fish fillet were transferred aseptically to a Stomacher bag (Seward Medical, London, UK) containing 225 mL of 0.1% peptone water (Merck, Cat No. 107228) and homogenised for 60 s using a Lab Blender 400, Stomacher at high speed (Stomacher, IUL Instrument, Spain).

Microbiological Media and Count For microbial count, 0.1-mL samples of serial dilutions (1:10, diluents, 0.1% peptone water; Merck, 107228) of fish homogenates were spread on the surface of agar plates. Total viable counts (TVC) were determined using plate count agar (Merck, 105463) after incubation for 24–48 h at 37 °C. Plate count agar was used for psychrotrophic bacteria and incubated at 7 °C for 10 days. Lactic acid bacteria were enumerated on MRS medium (Merck, 110660) and incubated at 37 °C for 48 h (Baumgart 1986). Results are expressed as a logarithm of colony forming units (log cfu) per gram of sample.

Sensory Analysis

The attributes of hot smoked fish were evaluated by a panel of five experienced judges on each week of sampling. Sensory evaluation was conducted in individual booths under controlled conditions of light, temperature and humidity. Sensory analysis was performed using the methods of Amerina et al. (1965). Smoked fish were assessed on the basis of appearance, odour, taste and texture characteristics using a nine-point descriptive scale. A score of 9–7 indicated "very good" quality; a score of 6.9–4.0, "good or acceptable quality"; and a score of 3.9–1.0, "unacceptable quality".

Physicochemical Analysis

The skin of smoked fish were removed for the physicochemical analysis and was homogenised using foot processor.

Chemical Analysis

One gram of each groups (three fillet) were mixed with 10 mL distilled water. The pH of the fish homogenate was measured using a digital pH meter Hanna pH 211 Microprocessor pH meter (HANNA Instruments, Michigan, USA), standardised at pH 4.0 and 7.0 (Erkan 2007). Total volatile basic nitrogen (TVB-N, mg/100 g fish flesh) was determined according to the method described by Antonacopoulos and Vyncke (1989). Trimethylamine nitrogen (TMA-N, mg/100 g fish flesh) was measured using the method of AOAC (1998), while the method used by Erkan and Özden (2008) was followed for the thiobarbituric acid (TBA) index values were expressed as mg of malondialdehyde (MDA)/kg of sample. The concentration of malondialdehyde was calculated from a standard curve using solutions of the MDA precursor (same molecular weight) 1,1,3,3-tetraethoxy-propane into distilled water after the addition of a quantity of TBA solution.

Colour Measurement

The colour of the fish samples was determined with the help of a Konica Minolta chromo meter (model CR 400/410; Minolta, Osaka, Japan). L^* (brightness), a^* (+a, red; −a, green) and b^* (+b, yellow; −b, blue) values were measured. The colorimeter was calibrated using white references (CR-A44). The fish muscles (three fillets) were homogenised and placed in glass petri dishes (12 cm diameter), and the colour measurement was repeated three times using different parts of the surface. The average of these values for L^*, a^* and b^* parameter was calculated (Gerdes and Santos Valdez 1991).

Statistical Analysis

All analytical determinations were at weeks 1, 2, 3, 4, 5, 6 and 7. Experiments were replicated twice on different occasions with different fish samples. The mean each sample for each group was analysed three times (n = replicate × group × three times; $n = 2 \times 3 \times 3$). Results are reported as mean values±standard deviation (SD). Data were subjected to analysis of variance. The Tukey's honestly significant difference procedure was used to test for differences between means ($P<0.05$) (Sümbüloğlu and Sümbüloğlu 2002).

Results and Discussion

Assessment of Microbiological Parameters

Changes in TVC of refrigerated hot smoked rainbow trout fillets during storage under vacuum packaging with or without thyme and garlic oil are shown in Fig. 1a. Low initial (week 1) TVC of hot smoked rainbow trout fillets of approximately 3.00 cfu/g (VP), 2.78 cfu/g (TO+VP) and 2.88 log cfu/g (GO+VP) indicates good fish quality, in agreement with results (3.3 log cfu/g) reported by Cakli et al. (2006) for vacuum-packaged rainbow trout fillets. At the end of shelf life after 7 weeks at 2 °C, respectively, levels of 6.65, 5.40 and 5.10 log cfu/g were reached. TVC is an important criterion for quality evaluation; the maximum recommended bacterial count for good-quality products is 5.7 log cfu/g, and the maximum recommended bacterial count for marginally acceptable quality products is 7 log cfu/g (ICMSF 1986). Considering these values, it is possible to say that TVCs of vacuum-packaged smoked rainbow trout (untreated) exceeded the microbiological limits of acceptability after 4 weeks of storage. This limit was not exceeded throughout storage in TO+VP and GO+VP samples. Thus, compared with the control samples (VP), a shelf life extension of 3 weeks was achieved for samples TO+VP and GO+VP, respectively, as determined by microbiological parameters (TVC). The thyme and garlic oil can be considered effectively inhibitory on the total aerobic flora.

Initial populations of psychrotrophic bacteria of VP, TO+VP and GO+VP samples were 2.88, 2.78 and 2.66 log cfu/g, respectively. Psychrotrophic bacteria count reached final counts of 7.47, 7.18 and 6.90 log cfu/g, respectively, for VP, TO+VP and GO+VP hot smoked trout samples (Fig. 1b). Interestingly, TO+VP and GO+VP smoked samples, on 5 weeks of storage, had significantly lower ($p<0.05$) counts than had the control samples. VP and TO+VP/GO+VP smoked trout samples exceeded the value of 6 log cfu/g for psychrotrophic bacteria count, considered as the upper acceptability limit for fish and fish products (Erkan 2007), after weeks 4 and 6 of storage, respectively. Thus, compared with the control samples (VP), a shelf life extension of 2 weeks was achieved for samples TO+VP and GO+VP, respectively, as determined by psychrotrophic bacteria counts.

Lactic acid bacteria (LAB) were dominant in vacuum-packed foods. Initial counts were approximately 1.50 log cfu/g (LAB), indicating a good hygiene of the marine environment from which the fish were caught, as well as good fishing practices and handling. Final populations of LAB (6.32, 5.94 and 5.24 log cfu/g) were recorded for treatments V, TO+VP and GO+VP, respectively (Fig. 1c). Giatrakou et al. (2008) and Kykkidou et al. (2009) reported that thyme–oregano oil treatment was effective in eliminating the growth of TVC and LAB in modified atmosphere packaged fish under refrigerated storage. Likewise, Kostaki et al. (2009) reported that the treatment of fresh fish fillets (sea bass) with a mixture of thyme oil reduced the final TVC and LAB counts by approximately 1 log, compared

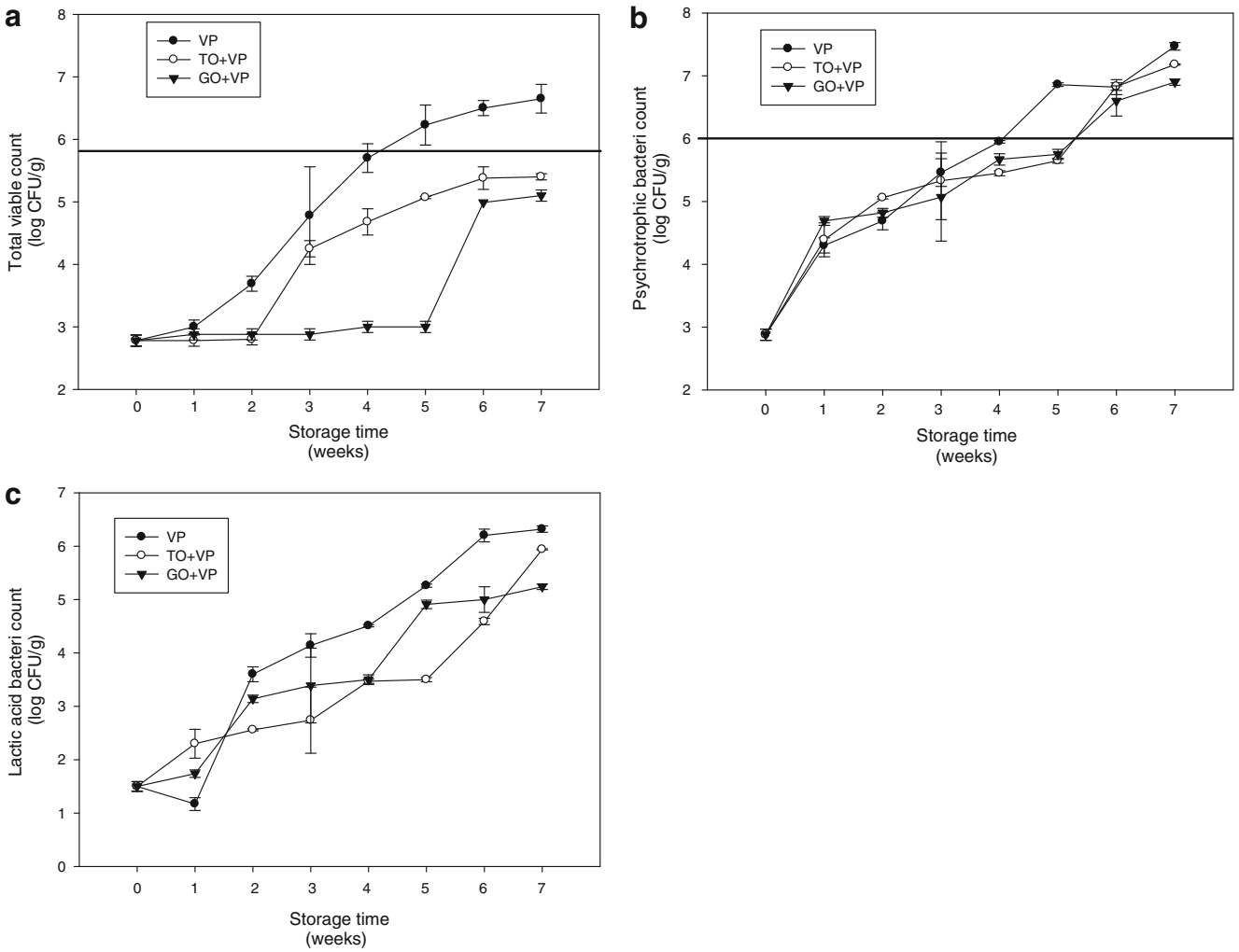

Fig. 1 Changes in total viable counts (**a**), psychrotrophic bacteria counts (**b**) and lactic acid bacteria counts (**c**) of hot smoked rainbow trout fillets during storage at 2 °C under vacuum packaging (*VP*), vacuum packaging plus thyme oil (1% *v*/*w*) (*TO*+*VP*) and vacuum packaging plus garlic oil (1% *v*/*w*) (*GO*+*VP*)

with control. They are also in agreement with those reported by Chouliara et al. (2007), who reported a reduction in growth of aerobic bacteria by 5 log cfu/g in chicken meat after 9 days of storage under 1% oregano oil and air packaging.

Assessment of Sensory Acceptance

The results of the sensory evaluation (overall appearance, texture, odour and taste) of smoked rainbow trout samples are presented in Fig. 2a–d. Overall appearance, texture, odour and taste scores for hot smoked samples stored under vacuum conditions, with thyme and garlic essential oil, showed a similar pattern of decreasing acceptability. The spoilage patterns described by the panelists were as follows: softening of texture before off-odours developed and presence of bitter and rancid off-flavours. Appearance score decreased as colour discolouration increased.

The limit of overall appearance was reached after 5 weeks for the control and after 7 weeks for GO+VP samples. The limit of acceptability for texture and odour was reached after 6 and 7 weeks of storage for control and thyme–garlic oil-treated samples, respectively. The limit of acceptability for taste was reached after 4 weeks for VP samples, while this limit was reached after 6 weeks for GO+VP and after 7 weeks for TO+VP samples. Based on appearance, texture, odour and taste scores, a shelf life of approximately 5 weeks for the VP samples, 7 weeks TO+VP samples and 6 weeks GO+VP may be expected. Autolytic enzymes can have a major impact on the loss of textural quality of smoked fish during the early stages of deterioration, but they did not produce the characteristic off-flavours and off-odours, which are typical of microbiological activity (Truelstrup et al. 1996). Lactic acid bacteria have been reported to be potential spoilage organisms in smoked fish products, and lactic acid bacteria are responsible off-flavours and off-odours (Leroi et al.

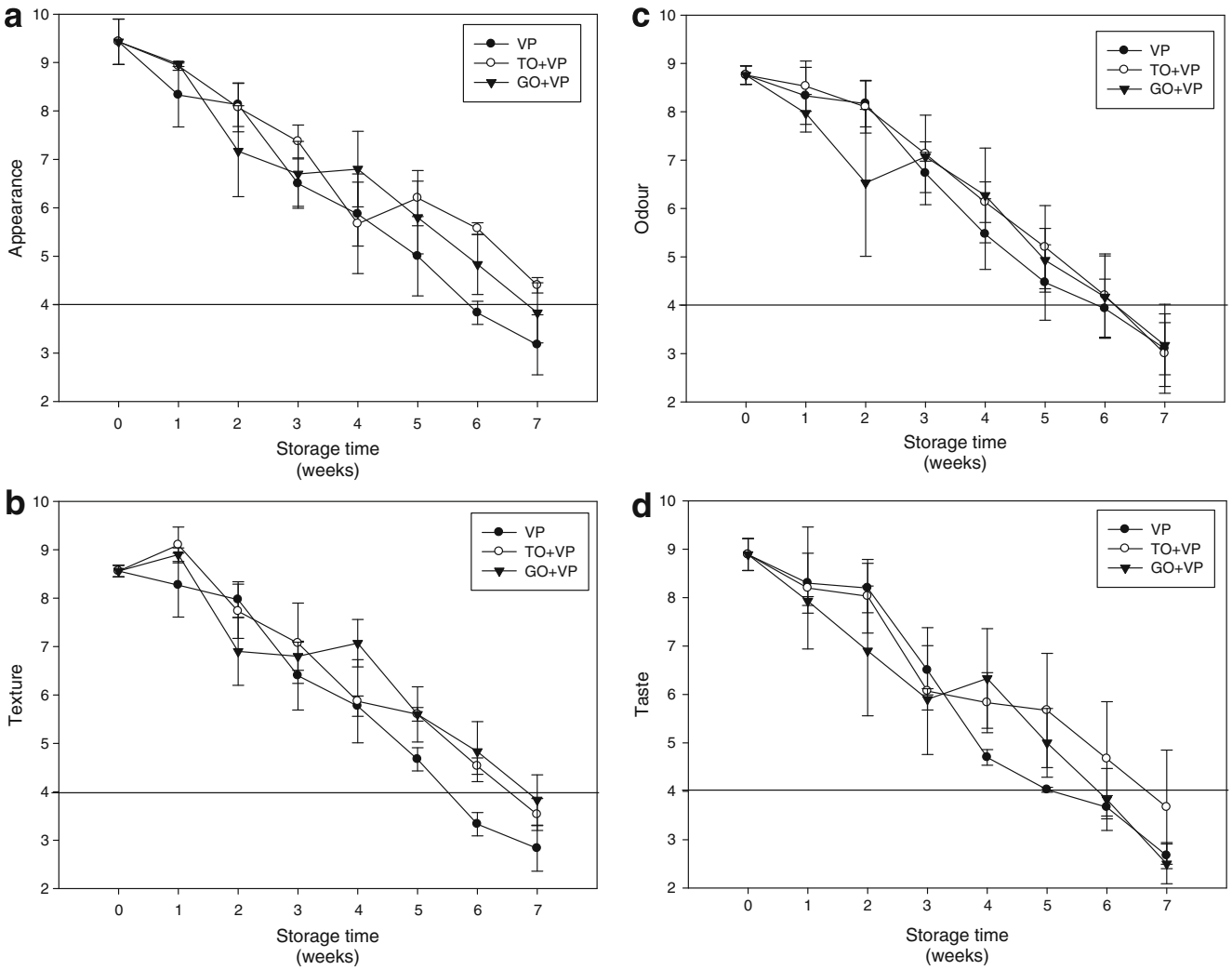

Fig. 2 **a**–**d** Changes in sensory analysis of hot smoked rainbow trout fillets during storage at 2 °C under vacuum packaging (*VP*), vacuum packaging plus thyme oil (1% *v*/*w*) (*TO*+*VP*) and vacuum packaging plus garlic oil (1% *v*/*w*) (*GO*+*VP*)

1998). Yanar et al. (2006) reported a shelf life for hot smoked catfish of 5 weeks, while Yanar (2007) reported a shelf life for wrapped hot smoked catfish stored at refrigerator temperature of 3.5 weeks. The shelf life of hot smoked vacuum-packaged rainbow trout stored in a refrigerator condition was found to be 5 weeks (Cakli et al. 2006).

The combination of vacuum and garlic–thyme oil resulted in a shelf life extension of approximately 1–2 weeks, attributed to the antimicrobial effects of the thyme oil phenolic components thymol and carvacrol, and garlic oil components, allicin, known to exert antimicrobial activity (Holley and Patel 2005). Likewise, Giatrakou et al. (2008), Kostaki et al. (2009), and Kykkidou et al. (2009) reported that the treatment of fresh fish (sword fish and sea bass) fillets with a mixture of oregano and thyme oil extended the shelf lives by approximately 5 days, compared with the control.

Assessment of Physicochemical Parameters

At the beginning of the storage period, pH values of hot smoked rainbow trout were determined as 5.83. pH values of control and treated samples showed increase and decrease during the storage. At the end of the storage period of 7 weeks, pH values of samples were found to be 5.58, 5.91 and 5.48, respectively (Fig. 3a). Similar results have been reported by Kolsarıcı and Özkaya (1998) for hot smoked trout under vacuum conditions. The pH first increases after decreases are in agreement with the findings of Erkan et al. (2007), for chub mackerel stored in vacuum.

The TVB-N may be considered as a quality index for fish. Its increase is related to the activity of spoilage bacteria and endogenous enzymes (Erkan et al. 2007). At the beginning of storage, the TVB-N value was 23.22 mg/100 g flesh, which was approximately similar to the values reported for hot smoked rainbow (18.55 mg/100 g), for cold

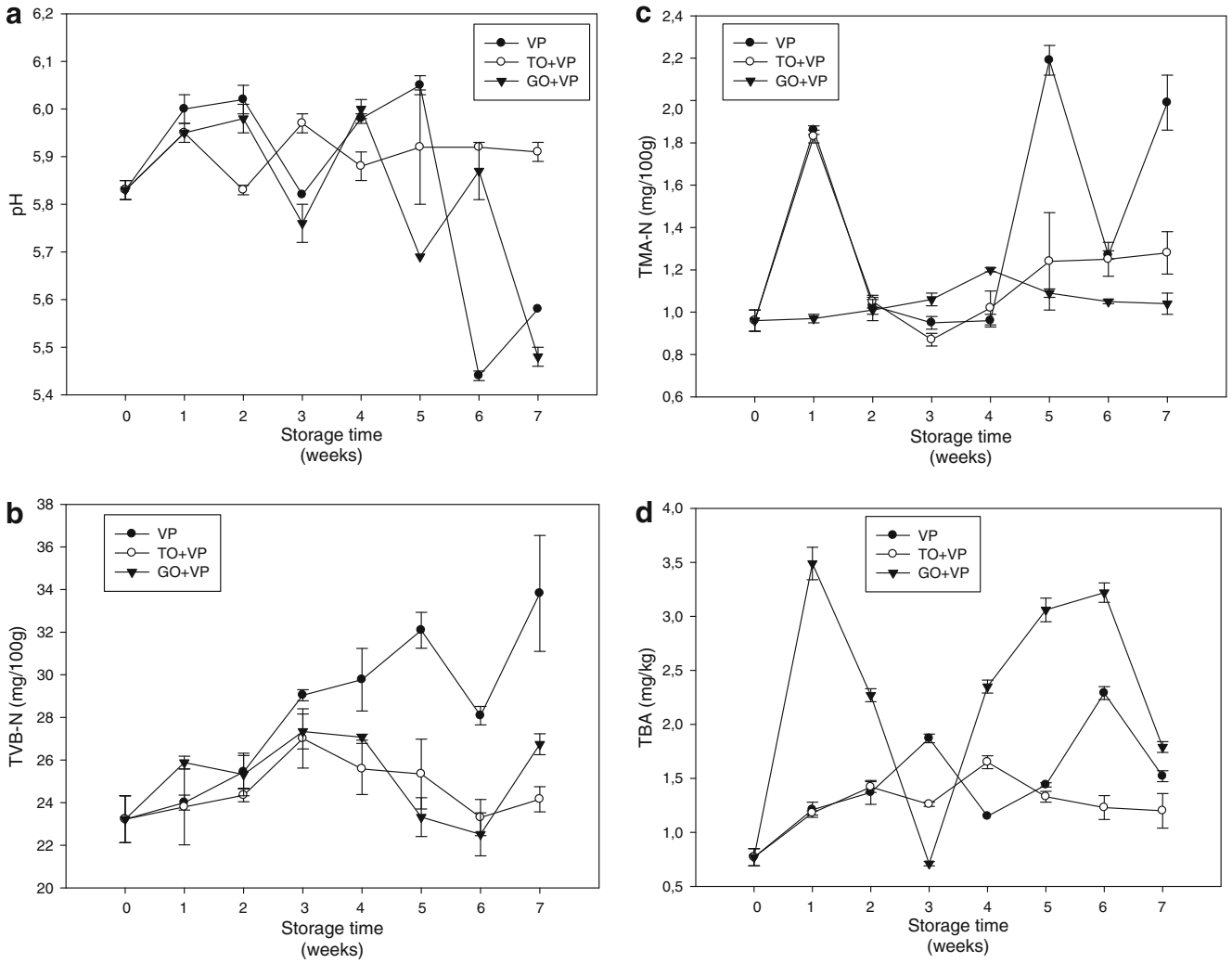

Fig. 3 Changes in pH (**a**), TVB-N (**b**), TMA-N (**c**) and TBA index (**d**) values of hot smoked rainbow trout fillets during storage at 2 °C under vacuum packaging (*VP*), vacuum packaging plus thyme oil (1% *v/w*) (*TO+VP*) and vacuum packaging plus garlic oil (1% *v/w*) (*GO+VP*)

smoked rainbow trout (20.00 mg/100 g) stored in a refrigerator (Kolsarıcı and Özkaya 1998) and for hot smoked catfish (17.67 mg/100 g) stored in a refrigerator, reported by Yanar (2007). As shown in Fig. 3b, the TVB-N value of samples untreated and treated with thyme and garlic essential oil vacuum-packaged trout rose to 33.82, 24.16 and 26.74 mg/100 g flesh by the end of the storage period. With regard to data on the limit of highest acceptable level of TVB-N for fresh fish, Huss (1988) proposed a TVB-N value of 30–35 mg/100 g whilst, recently, Giménez et al. (2002) proposed a value of 25 mg/100 g as the onset of spoilage for rainbow trout. In the present study, the TVB-N level showed fluctuations during storage, indicating that TVB-N could not be a good indicator of rainbow trout quality, as also proposed by Chytiri et al. (2004) and Rezaei et al. (2008) for rainbow trout. On the basis of the present data (total viable count and sensory evaluation), a similar TVB-N limit value of 32.09 mg/100 g may be proposed for the initiation of vacuum-packaged hot smoked trout spoilage. Hot smoked trout samples VP, TO+VP and GO+VP exceeded the proposed (in our study) upper TVB-N acceptability limit on weeks 5, 7 and 7, respectively, values that could be assigned as the physicochemical (using TVB-N as a parameter) shelf life of hot smoked trout samples under the experimental conditions of the present study. The inhibition of TVB-N production in second and third groups smoked rainbow trout samples may be attributed to the effects of VP and/or with the combined synergistic effect of thyme and garlic oil, the latter known to possess antibacterial properties due to its phenolic (carvacrol and thymol) and organosulphur (allicin) constituents (Burt 2004).

TMA is produced by the decomposition of trimethylamine oxide due to bacterial spoilage and enzymatic activity (Tülsner 1994). The initial TMA-N values of samples were found 0.96 mg/100 g. TMA-N values of

control and TO+VP/GO+VP samples increased significantly ($p<0.05$) with storage time; by the end of the storage period (week 7), however, samples vacuum-packaged with thyme and garlic oil reached a significantly ($p<0.05$) lower TMA-N value of 1.28 and 1.04 mg/100 g, respectively, in comparison with the samples of controls, which attained higher levels of 1.99 mg/100 g (Fig. 3b). Lower production of TMA-N in TO+VP and GO+VP hot smoked samples may be due to the antibacterial properties of thyme and garlic oil. TMA-N is not a particularly useful indicator of fresh water fish (trout) freshness (Tülsner 1994). Given the great variation in TMA-N acceptability limits for various fish species reported, and TMA-N values shown in Fig. 3, as well as data based on sensory (overall acceptance) scores (5 weeks) and microbiological (TVC) data (5 weeks), a more realistic TMA-N limit of acceptability for hot smoked rainbow trout, of ca. 2.00 mg/100 g, may be proposed. Similarly, low TMA-N values have been reported for hot smoked rainbow trout Cakli et al. (2006). On the basis of this limit, VP, TO+VP and GO+VP hot smoked rainbow trout fillets exceeded this value on weeks 5, 7 and 7, respectively, which could be used to mark the shelf life of hot smoked trout fillets. Lower production of TMA-N in TO+VP and GO+VP smoked fish samples may be due to the antibacterial properties of thyme and garlic oil. Interestingly, vacuum packaging thyme–garlic oil treatment (combination of packaging and oil) effectively controlled the population of TVC to less than 7 log cfu/g during refrigerated storage of smoked fish (Fig. 1a).

Lipid oxidation is a major quality problem especially in fatty marine species. It leads to the development of off-odours and off-tastes in edible oils and fat containing foods, known as oxidative rancidity. The TBA index value is an

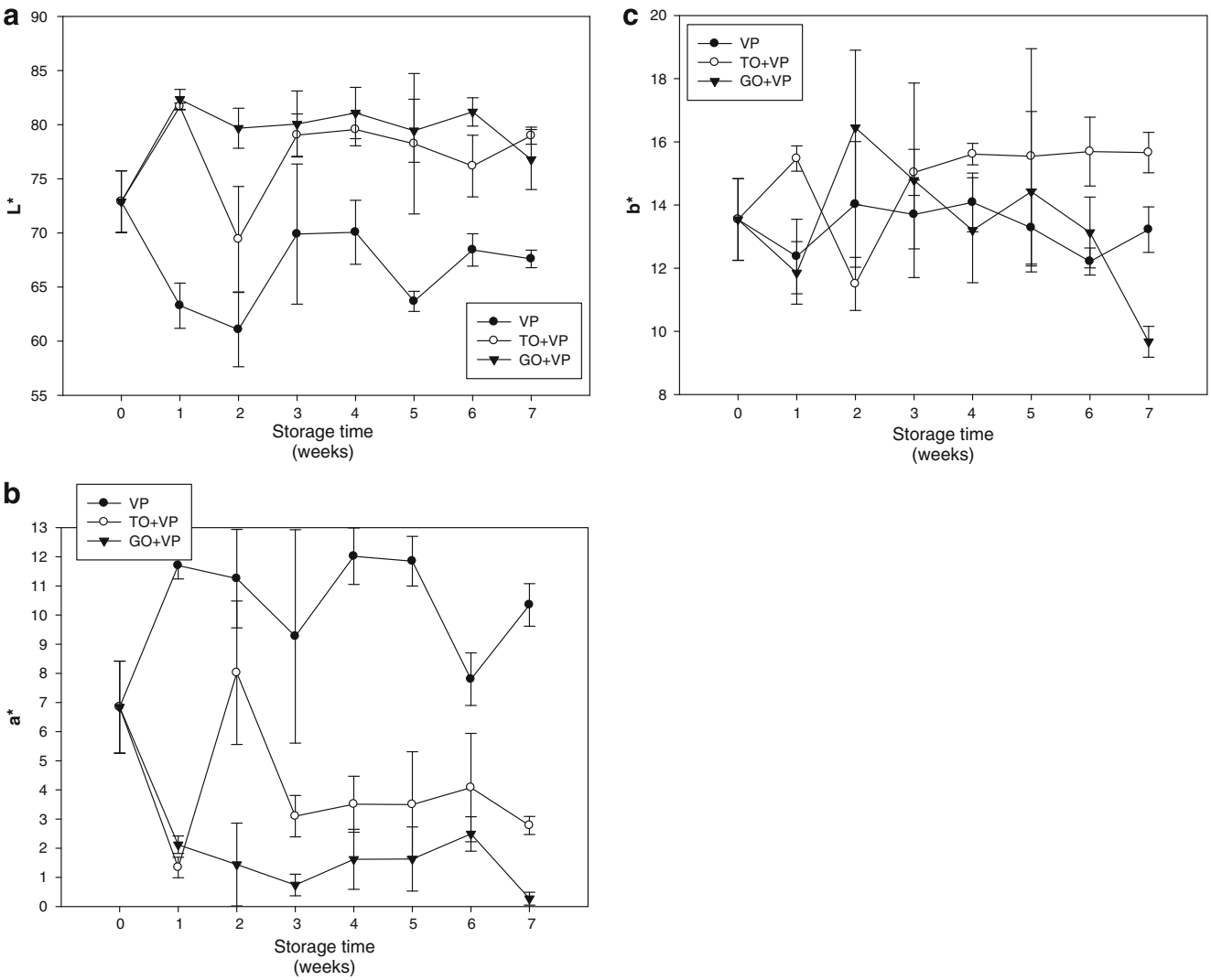

Fig. 4 Changes in L^* (**a**), a^* (**b**) and b^* (**c**) values of hot smoked rainbow trout fillets during storage at 2 °C under vacuum packaging (*VP*), vacuum packaging plus thyme oil (1% *v*/*w*) (*TO+VP*) and vacuum packaging plus garlic oil (1% *v*/*w*) (*GO+VP*)

index of lipid oxidation measuring MDA content. MDA is formed through hydroperoxides, which are the initial reaction products of polyunsaturated fatty acids with oxygen (Rezaei et al. 2008). Changes in TBA values for VP, TO+VP and GO+VP hot smoked trout samples during storage are shown in Fig. 3d. The initial TBA index value for hot smoked rainbow trout fillets was 0.77 mg MDA/kg in agreement with Yanar (2007) and Cakli et al. (2006) who reported a TBA index value for hot smoked tilapia and rainbow trouts of 0.84 and 1.4 mg MDA/kg. In the case of vacuum-packaged smoked fish, the TBA index value increased to a maximum during storage up to the third week and decreased. Similar results were determined in TO+VP and GO+VP samples. Lower production of TBA index in TO+VP hot smoked samples may be due to the antioxidant properties of thyme oil. The decrease in TBA index values may represent the breakdown of the malondialdehyde to tertiary degradation. Similar results have been obtained in hot smoked tilapia (Yanar et al. 2006) and hot smoked trout Cakli et al. (2006). The TBA values for TO+VP and GO+VP were significantly lower ($P<0.05$) than those for VP, indicating that the TO and GO treatments used in TO+VP and GO+VP inhibited lipid oxidation. The use of thyme oil to protect muscle foods against oxidation has been reported in the literature. Mariutti et al. (2008) and Erkan and Bilen (2010) observed that a garlic oil and thyme oil was an effective means of controlling lipid oxidation in chicken and fish meat, as reflected in thiobarbituric acid reactive substance values.

Colour is an important parameter (Du and Sun 2008). Colour values of the samples can be seen in Fig. 4a–c. At the beginning of the storage, L^* values of VP, TO+VP and GO+VP hot smoked samples were determined as 72.89±2.85. In the TO+VP and GO+VP samples, L^* values increased according to time of storage. At the end of the storage period of 7 weeks, L^* values reached 78.98±0.79 and 76.78±2.77 for TO+VP and GO+VP, respectively. According to the results on the seventh week of storage, vacuum-packaged hot smoked fish flesh became much lighter. At the beginning of the storage period, a^* values for samples were determined as 6.84±1.58. At the end of the storage period of 7 weeks, a^* values of VP samples increased to 10.35±0.73 and a^* values of TO+VP and GO+VP samples decreased to 2.78±0.31 and 0.27±0.22, respectively. b^* values of VP and TO+VP samples were increased at the end of the storage, whereas GO+VP were decreased at the end of the storage. Chouliara et al. (2007) reported similar in L^*, a^* and b^* values for oregano essential oil and modified atmosphere packaging in fresh chicken meat during 12 days of storage.

Conclusions

In the present study, a shelf life of 7 weeks was recorded for hot smoked trout treated with thyme and garlic oil in cold storage, compared with a shelf life of 5 weeks for the untreated hot smoked rainbow trout based on sensory, chemical and microbiological evaluation.

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