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Effect of thyme essential oil and packaging treatments on chemical and microbiological properties of fresh rainbow trout (*Oncorhynchus mykiss*) fillets during storage at refrigerator temperatures

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In this research, the effect of thyme essential oil (EO) and packaging treatments (vacuum and modified atmosphere) on fresh rainbow trout fillets during storage for a period of 18 days at 4°C was investigated. Fillets were subjected to microbiological (total viable count (TVC), *Pseudomonas,* lactic acid bacteria, Enterobacteriaceae) and chemical [pH, thiobarbituric acid reactive substances (TBARS), total volatile basic nitrogen (TVB-N)] analyses on certain days (0, 3, 6, 9, 12, 15, 18th) of storage. TBARS and TVB-N values increased in the duration of storage. Bacterial growth was inhibited in samples with increased concentration of thyme oil due to the antimicrobial activity of thyme oil. Overall, the combined use of thyme EO (0.8%, v/w) and modified atmosphere packaging (MAP) showed a synergistic effect for shelf-life extension of rainbow trout fillets.

Key words: Modified atmosphere packaging (MAP), rainbow trout, shelf life, thyme essential oil, vacuum.

INTRODUCTION

Fish farming has developed into a highly productive and efficient industry for production of animal protein (Rora et al., 2003). Rainbow trout (*Oncorhynchus mykiss*) is the mostly cultured fish species all over the world (Emre and Kürüm, 1998) and also in Turkey with the total production in 2006 as 57.659 tons (TUİK, 2006; İzci et al., 2009). Consumption of fresh fish, related to various reasons has become difficult, therefore aquaculture industry moved towards extending the shelf life of fish products. Microorganisms can reproduce in the muscle tissue of aquatic products because these are but also has rich nutrient content (Babadoğan, 1998). Rainbow trout (*O. mykiss*) is a fish belonging to the Salmonidae family, a species with high commercial value and much appreciated by European consumers (Cakli et al., 2006). Preservation methods for rainbow trout are smoking (Kolsarıcı and Özkaya, 1998; Cakli et al., 2006), packaging (Gimènez et al., 2002; Aras Hisar et al.. 2004), preservation by the addition of nitrite or potassium nitrate (Lyhs et al., 1998), combination of sodium acetat, sodium lactate, and sodium citrate (Kılınç et al., 2009). Vacuum packaging method is a type of passive modified atmosphere. After placing the food in a suitable packaging material in this operation, the air in the package is emptied by vacuum and a hermetic closure (air tight) is made. This method is usually used for preservation of meat products. Even in vacuum packaging, a very small amount of O_2 will remain and this low percentage of O_2 in the package is used by aerobic microorganisms, and CO_2 is produced. In these types of products, the bacterial growth and oxidation the product is prevented as air is not in the package (Keleş, 1998; Gülyavuz and Ünlüsayın, 1999; Kılınc and Çaklı, 2001; Çarbaş, 2008). Thus numerous studies have been

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conducted on the preservation of vacuum on fish and fish products for salmon (Hansen et al., 1998; Leroi et al., 1998; Leroi et al., 2000; González-Rodríguez et al., 2002; Dondero et al., 2004; Jónsdóttir et al., 2008), sea bream (Chouliara et al., 2004), swordfish (Muratore and Licciardello, 2005), chub mackerel (Stamatis and Arkoudelos, 2007a), ascidia (Stamatis et al., 2008), mackerel (Park et al., 2009), catfish (Rodríguez et al., 2009), and mahi sefid (Zolfaghari et al., 2010). Modified atmosphere packaging (MAP), is a preservation method use to extent shelf-life of fish and fish products (Özoğul et al., 2006). In modified atmosphere packaging, elimination of oxygen from inside the package and filling with different concentrations of CO_2 and N_2 is done, however, refrigerated storage conditions for aerobic microorganisms, proteolytic bacteria, yeast and mold growth is inhibited (Swiderski et al., 1997; Gülyavuz and Ünlüsayın, 1999; Kılınç and Çaklı, 2004). There are many MAP studies related to shelf life extension of fish and fishery products for hake (Pastoriza et al., 1996,1998), cod (Debevere and Boskou, 1996; Wang et al., 2008), catfish (Göktepe and Moody, 1998), mullet (Pournis et al., 2005), bass (Torrieri et al., 2006), chub mackarel (Erkan et al., 2007; Goulas and Kontominas, 2007), sardines (Stamatis and Arkoudelos, 2007b), eel (Arkoudelos et al., 2007), mussels (Goulas, 2008), and swordfish (Pantazi et al., 2008).

Essential oils (EOs) are aromatic oily liquids obtained from plant material. Extracts from oregano, thyme, rosemary, clove, sage and mint are some of the EOs that have been used both to improve the sensory characteristics and extend the shelf life of foods. A number of EOs and some of their components have been reported to have antimicrobial activity against a wide range of spoilage and pathogenic bacteria (Burt, 2004; Lambert et al., 2001). Thyme contains high concentrations of phenolic compounds including carvacrol, thymol, p-cymene and ɣ-terpinene (Marino et al.,1999). The aim of this study was to determine the combined effect of thyme EO and MAP and vacuum packaging in extending refrigerated shelf life of trout fillets.

MATERIALS AND METHODS

Preparing samples

Fish material, rainbow trouts (250±25 g) were obtained from Ataturk University Agricultural College Fisheries Department's Rainbow Trout Breeding And Research Center. Fish were carried to the laboratory and washed with tap water. A total of 72 fish samples were eviscerated, stored until rigor had resolved and then filleted; 144 fillets in total (Robb et al., 2002). Fillets were washed again to remove blood and mucous remains. All filleted samples including the control were packaged in 15x25 cm thickness Polyethylene/Polyamide (PA/PE) (3-seal bags GB 70) having an O_2 permeability of 40 cm^3/m^2/day.atm.23°C; N_2 permeability of 24 cm^3/m^2/day.atm.23°C; CO_2 permeability of 145 cm^3/m^2/day.atm.23°C and a water vapour permeability of < 3 g/m^2/day.atm.23°C); obtained from the firm Südpack Verpackungen GmbH+Co (Germany).

Treatment on fish samples

Thyme essential oil was added to the surface of the two lots of filleted samples using a micropipette in appropriate volumes (two sides) so as to achieve final 0.4 and 0.8% (v/wt) EO concentrations. Thyme essential oil was added undiluted using a micropipette. In all treatments (given below), the antimicrobials were massaged onto the product, so as to get even distribution of the oil using gloved fingers (to avoid cross-contamination of samples and also transmission of food poisoning organisms).

The treatments included: control samples vacuum packaged (CV), control samples modified atmosphere packaged-50% CO_2+50% N_2 (CM), vacuum packaged with added thyme EO 0.4% (v/w) (V1), (modified atmosphere packaged with added thyme EO 0.4% (v/w) (M1), vacuum packaged with added thyme EO 0.8% (v/w) (V2) and (modified atmosphere packaged with added thyme EO 0.8% (v/w) (M2). Each group included 24 fillets. Rainbow trout fillets were stored under refrigeration (4±1°C) and were subjected to microbiological (total viable counts, *Pseudomonas*, lactic acid bacteria, Enterobacteriaceae) and chemical (pH, thiobarbituric acid reactive substances-TBARS, total volatile base nitrogen-TVB-N) analyses on certain days (0, 3, 6, 9, 12, 15 and 18^{th} days) of storage.

Microbiological analysis

A sample (25 g) was taken from each group, transferred aseptically into a stomacher bag containing 225 ml of 0.1% peptone water and was homogenized for 60 s in a Stomacher (Lab Stomacher Blender 400-BA 7021 Sewardmedical, England) at room temperature. For microbial analyses, 0.1 ml samples of serial dilutions (1:10, diluent, 0.1% peptone water) were inoculated on to proper agar plates. Total viable counts (TVC) were determined on plate count agar (PCA, Merck 1.05463.0500) which were incubated at 30°C for 3 days. *Pseudomonads* were determined using cetrimide fusidin cephaloridine agar (CFC, Pseudomonas Agar Base-Oxoid CM0559 + CFC Selective Agar Supplement-Oxoid SR0103) after incubation at 25°C for 2 days. Lactic acid bacteria (LAB) were enumerated using de Man Rogosa Sharpe agar (MRS, de Man, Rogosa Sharpe Agar Oxoid CM0361) which was incubated at 30°C for 2 days. For Enterobacteriaceae, , Violet Red Bile Dextrose (VRBD) Agar Merck 1.10275.0500) which was incubated at 30°C for 2 days was used.

Chemical analysis

Total volatile basic nitrogen (TVB-N) was determined according to the methods of Malle and Tao (1987). TVB-N contents were expressed as mg 100/g fish muscle. Thiobarbituric acid reactive substance (TBARS) was determined according to the method of Lemon (1975) and Kılıç and Richards (2003). TBARS content was expressed as µmol Malondialdehyde (MDA)/kg fish muscle. pH was determined according to the method of Gökalp et al. (1995).

Statistical analysis

Experiments were replicated twice on two separate occasions with different fish samples. Analyses were run in duplicate for each replicate. All obtained data from this study were subjected to analysis of variance (ANOVA), and followed by Duncan's multiple range test to determine significant differences among means

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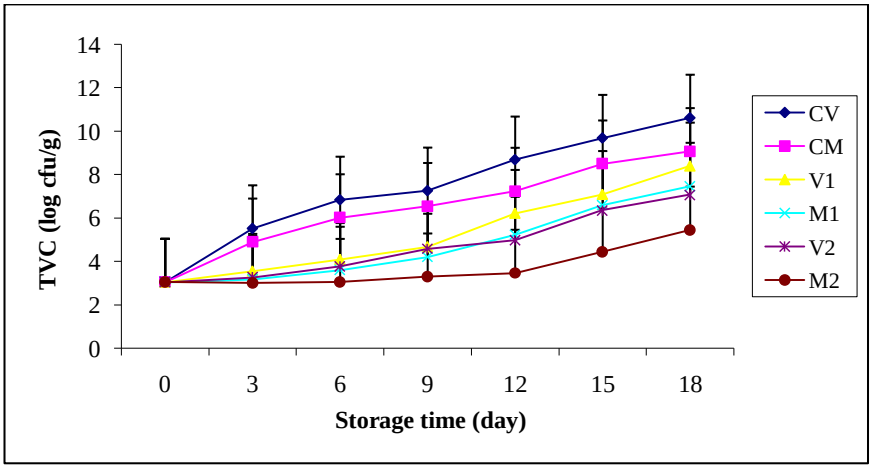

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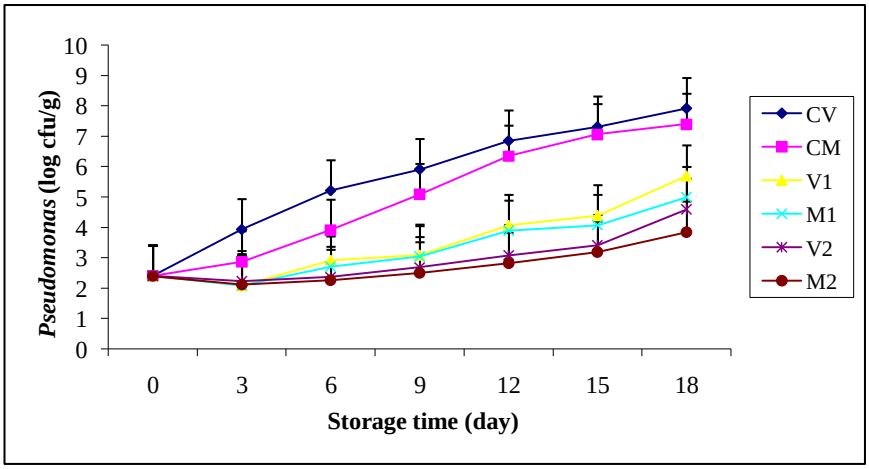

Figure 1. TVC (a), *Pseudomonas* (b), lactic acid bacteria counts (c) and Enterobacteriaceae counts (d) changes of treatment with thyme essential oil (0.4% and 0.8 v/w) rainbow trout fillets during cold storage in vacuum and MAP conditions at 4°C.

at α = 0.05 level, using SPSS (1999).

RESULTS

Microbiological changes

Changes in TVC of refrigerated fresh rainbow trout fillets during storage under vacuum and modified atmosphere packaging with or without thyme oil are shown in Figure 1a. The initial (day 0) TVC (Figure 1a) of rainbow trout fillets was 3.03 log cfu/g. CV, CM, V1, M1, V2 rainbow trout fillets exceeded the value of 7 log cfu/g for TVC, which was considered as the upper acceptability limit for fresh marine species (ICMSF, 1986) on days 9, 12, 15, 18 and 18 of storage, respectively. This limit was not exceeded throughout storage in M2. At the end of storage period of 18 days, CV, CM, V1, M1, V2 and M2 respectively, levels of 10.61, 9.07, 8.40, 7.47, 7.04 and 5.45 log cfu/g were reached.

The initial (day 0) *Pseudomonas* (Figure 1b) of rainbow trout fillets was 2.40 log cfu/g. *Pseudomonas* reached final (day 18) counts of approximately 7.92 log cfu/g for CV samples, whereas lower populations of approximately 3.85 log cfu/g were recorded for M2 samples. Initial counts were 2.0 log cfu/g (LAB) (Figure 1c) and 2.20 log cfu/g (Enterobacteriaceae) (Figure 1d). At the end of storage period, populations of LAB (7.39, 6.26, 5.42, 5.09, 4.95 and 4.04 log cfu/g) and Enterobacteriaceae (8.68, 7.67, 7.09, 6.60, 6.07 and 4.45 log cfu/g) were recorded for treatments CV, CM, V1, M1, V2 and M2, respectively.

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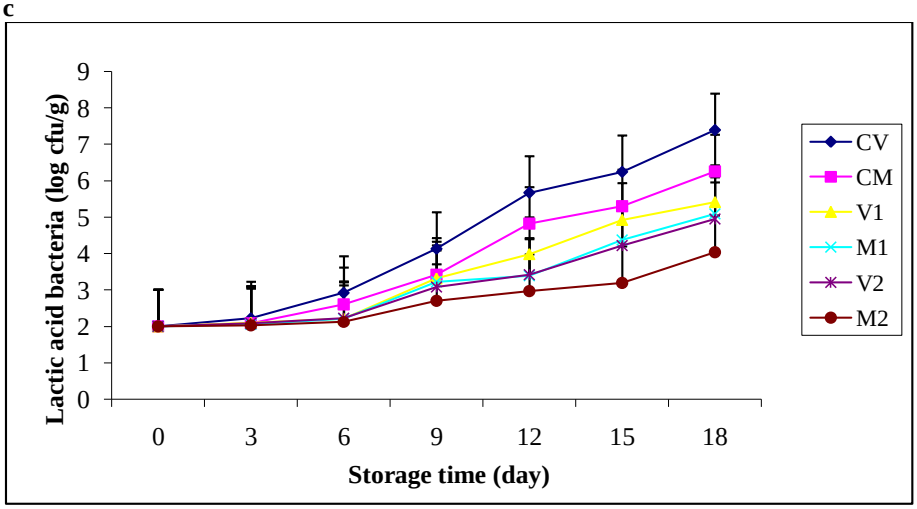

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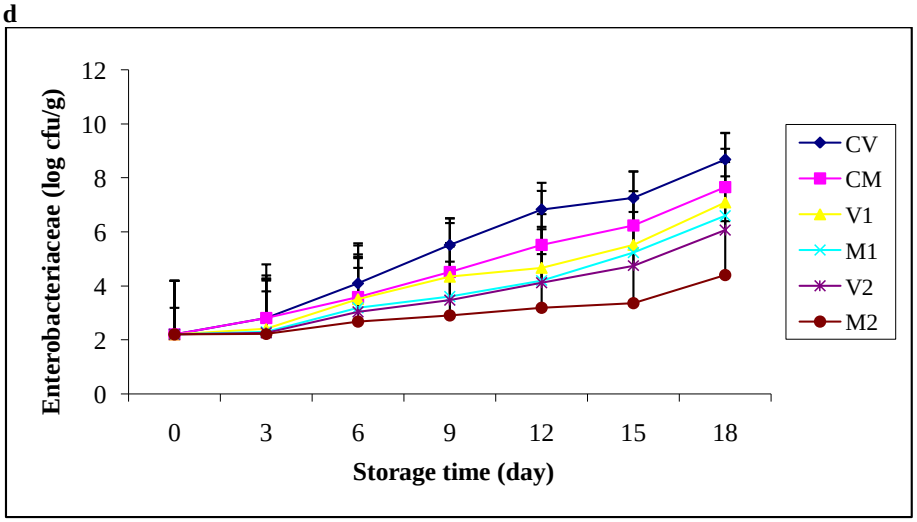

Figure 1. Continued.

Chemical changes

TVB-Nitrogen

The amount of TVB-N is an important criteriation in determining freshness of fish and fish products and TVB-N values increase in paralel to spoilage (Köse and Koral, 2005). The initial (day 0) TVB-N values (Figure 2a) of rainbow trout fillets were 16.43 mg/100g. CV, CM, V1 and M1 rainbow trout fillets exceeded the value of 25 mg/100 g, the upper acceptable limit TVBN value of 25 mg N/100 for rainbow trout, suggested by Aras Hisar et al. (2004) on days 9, 18, 15 and 18 of storage, respectively. This limit was not exceeded throughout storage in V2 and M2.

Lipid oxidation

Oxidative rancidity may become a problem if higher than normal levels of oxygen are used. Rancidity due to oxidation of polyunsaturated fatty acids (PUFA) in some fish may be a problem in modified atmosphere with O_2 (Aras Hisar et al., 2004; Finne, 1982; Stammen et al., 1990).

Initial TBARS values (Figure 2b) were 2.5 µmol malondialdehyde (MDA)/kg. At the end of storage period, TBARS values 8.18, 7.85, 6.09, 4.93, 4.37 and 3.69 µmol malondialdehyde (MDA)/kg were recorded for treatments CV, CM, V1, M1, V2 and M2, respectively.

pH

pH value of fish meat usually ranges from 5.7 to 6.6. Fresh fish is close to neutral pH, but after death the lactic acid is formed which firstly falls and then rises again with spoilage (Bilgin, 2003). Initial pH values of rainbow trout fillets (Figure 2c) were 6.30. At the end of storage period,

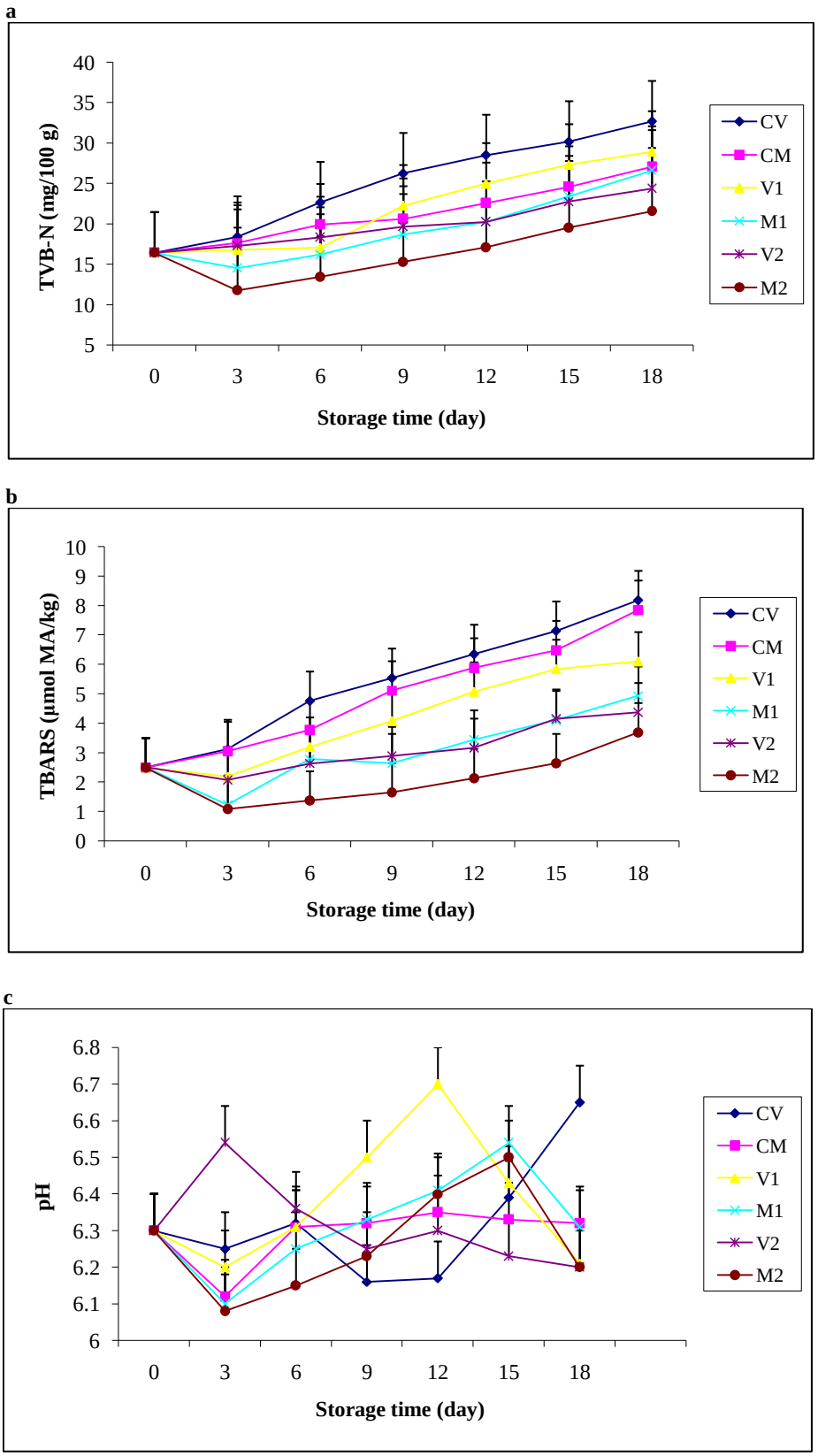

Figure 2. TVB-N (a), TBARS (b), and pH (c) changes of treatment with thyme essential oil (0.4% and 0.8 v/w) rainbow trout fillets during cold storage in vacuum and MAP conditions at 4°C.

pH values for treatments CV, CM, V1, M1, V2 and M2 were recorded as 6.65, 6.32, 6.22, 6.32, 6.20 and 6.20 respectively.

DISCUSSION

TVC is an important criterion for quality evaluation. The initial TVC numbers was 3.03 log cfu/g while this value increased during storage time in all groups. Similar results were observed by many other researchers (Chytiri et al., 2004; Caklı et al., 2006; Frangos et al., 2010; Pyrgotou et al., 2010) for rainbow trout. Bacterial growth was inhibited in samples with increased concentration of thyme oil due to the antimicrobial activity of thyme oil. The possible synergistic effect of MAP and thyme oil delayed microbial growth and suppressed final counts of spoilage microorganisms in rainbow trout under MAP. Bacterial growth of MAP samples were lower than vacuum packaged samples probably because of CO_2 exist in MAP. The application of MAP has been previously reported to extend the shelf life of hake (Pastoriza et al., 1996,1998), cod fillets (Debevere and Boskou, 1996; Lauzon et al., 2009), Baltic herring fillets (Randell et al., 1997), rainbow trout (Aras Hisar et al., 2004) and eel (Arkoudelos et al., 2007). *Pseudomonas* spp. and *Shewanella putrefaciens* were early recognised as putative spoilage inducers in fish muscle and have since then been found in various fish species from fresh and marine waters as well as in other foods (Castell et al., 1997; Macdonell and Colwell, 1985; Reynisson et al., 2009). Similar initial *Pseudomonas* (day 0) were reported for rainbow trout by Mexis et al. (2009), Frangos et al. (2010) and Pyrgotou et al. (2010). LAB are facultative anaerobic bacteria that can grow under both anaerobic and aerobic conditions (Jay, 1986). LAB were also part of the natural microflora of fresh rainbow trout fillets (Figure 1c). The initial LAB counts was 2.0 log cfu/g while this values increased during storage time in all groups.

Enterobacteriaceae, a hygiene indicator, were also part of the microflora of fresh rainbow trout. The initial Enterobacteriaceae counts was 2.20 log cfu/g while this value increased during storage time in all groups. Similar findings were found for rainbow trout (Mexis et al., 2009; Oğuzhan and Angiş, 2012), and sardine (Can, 2011).

The TVB-N may be considered as a quality index for fish and its increase is related to the activity of spoilage bacteria and endogenous enzymes (Erkan et al., 2007). The initial (day 0) TVB-N numbers was 16.43 mg/100 g while this values increased in the duration of storage time in all groups. Similarly, TVB-N values have been reported for sea bream (Goulas, 2008), swordfish (Kykkidou et al., 2009), and rainbow trout (Mexis et al., 2009).

TBA value is an index of lipid oxidation measuring malondialdehyde (MDA) content (Goulas, 2008). Initial TBARS values were 2.5 µmol malondialdehyde (MDA)/kg. Increased TBARS values was observed in the duration of storage time in all groups. Similarly, TBARS values have been reported for rainbow trout (Aras Hisar et al., 2004; Finne, 1982). At the beginning of the storage period, pH values of rainbow trout fillets were determined as 6.30. pH values of the control and treated samples showed increase and decrease during storage. Similarly, pH values have been reported for sea bream (Goulas, 2008), and rainbow trout (Oğuzhan and Angiş, 2012). However, pH values of modified atmosphere packaged control group were lower than vacuum packaged samples. This result can be related to conversion of CO_2 to carbonic acid. According to microbiological data, the shelf life of CV, CM, V1, M1 and V2 were 9, 12, 15, 18 and 18 days respectively. The shelf life of the modified atmosphere packaged with added thyme EO 0.8% [v/w] rainbow trout (M2) was more than 18 days. The present study shows that the combination of thyme EO (0.8%, v/w) and MAP (50% CO_2+50% N_2) was very effective in extending the shelf life of fresh rainbow trout fillets.

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