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Combined effect of an oxygen absorber and oregano essential oil on shelf life extension of rainbow trout fillets stored at 4 °C

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ABSTRACT

In the present study the combined effect of an O_2 absorber and oregano essential oil (0.4% v/w) on shelf life extension of rainbow trout fillets (*Onchorynchus mykiss*) stored under refrigeration (4 °C) was investigated. The study was based on microbiological [TVC, *Pseudomonas* spp., Lactic Acid Bacteria, H_2S-producing bacteria including *Shewanella putrefaciens*, Enterobacteriaceae and *Clostridium* spp.), physicochemical (pH, PV, TBA, TVBN and Drip loss) and sensory (odor, taste) changes occurring in the product as a function of treatment and storage time. Aerobically-packaged rainbow trout fillets stored at 4 °C were taken as control samples. Results showed that TVC exceeded 7 log cfu/g on day 4 of storage for control samples, day 7–8 for samples containing oregano oil, day 9 for samples containing the O_2 absorber and day 12–13 for samples containing the O_2 absorber and oregano oil. *Pseudomonas* spp., Enterobacteriaceae and LAB were only partially inhibited by the O_2 absorber and/or the oregano oil. In all cases the inhibition effect was more pronounced when the combination of O_2 absorber with oregano essential oil was used. pH decreased from an initial value of 6.65–6.09 and subsequently increased to 6.86 due to formation of protein decomposition products. % Drip loss ranged between 7% and 11–12% at the end of the product shelf life. PV values ranged between 11.4 and 27.0 meq O_2/kg oil while malondialdehyde (MDA) ranged between 9.6 and 24.5 mg/kg. TVBN ranged between 10.6 and 54.6 mg/kg at the time of sensory rejection. Sensory shelf life was 4 days for the control samples, 7–8 days for samples containing oregano oil, 13–14 days for samples containing the O_2 absorber and 17 days for samples containing the O_2 absorber plus oregano oil.

1. Introduction

Several species of marine and freshwater fish such as sea bream (*Sparus aurata*), sea bass (*Dicentrarchus labrax*) and rainbow trout (*Onhcorynchus mykiss*) are being farmed in Greece and other Mediterranean countries in order to meet the increasing demand of consumers for fresh fish (Urch, 1994). Of the freshwater fish species, rainbow trout (*Onhcorynchus mykiss*) is being farmed mainly in the river waters of North Western Greece and is sold either as whole fish or in the form of fish fillets in retail markets (Chytiri et al., 2004). Additionally, trout fillets mainly smoked and vacuum packaged are being exported to various European countries and consumed without further heat treatment.

Fresh fish (including rainbow trout) are more susceptible compared to red meats and chicken. This is due to large amounts of free amino acids and volatile nitrogen bases and a higher final pH limiting the shelf life of the product (Ashie et al., 1996). Enzymatic and chemical reactions are usually responsible for the initial loss of freshness, while microbial activity is responsible for subsequent spoilage (Mohan et al., 2008). Both the economic drive and consumers' demand for mildly preserved products have resulted in the use of new technologies that will maintain the quality of fish.

Active packaging refers to the incorporation of specific additives into packaging film or container with the aim of maintaining quality and extending product shelf life (Day, 1989). The most widely used active packaging concepts are those developed to scavenge oxygen and were first commercialized in 1970 by Mitsubishi Gas Chemical Company (Ageless®, Japan). The purpose of the oxygen scavenger is to create a low O_2 atmosphere within the pack preventing deterioration through oxidation and growth of aerobic microorganisms (Mohan et al., 2008). Ageless® is the most common O_2 absorber system based on iron (Fe^{2+}) oxidation (Nakamura and Hoshino, 1983). The sachets are designed to reduce O_2 levels to less than 0.01% (Labuza, 1987). Oxygen absorbers have been used effectively to prevent discoloration of cured meats, rancidity problems in high-fat foods, mold growth in high moisture

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bakery products etc. (Berenzon and Saguy, 1998). Besides the advantages, the use of O_2 absorbers has also certain disadvantages. An anoxic environment in the case of foods with water activity greater than 0.92 may enhance the growth of anaerobic pathogens including *Clostridium botulinum* and thus may introduce health risks if the temperature is not kept below 3 °C (Mohan et al., 2008). Recent studies have demonstrated both antimicrobial activity of essential oils (EOs) against foodborne pathogens (Burt, 2004) and extension of the shelf life of foods (Botsoglou et al., 2003; Chouliara and Kontominas, 2006). Oregano is one of the most characteristic spices of the Mediterranean cuisine, obtained by drying leaves and flowers of *Origanum vulgare*. It is well known for its antioxidative and antimicrobial activity (Botsoglou et al., 2003,) mainly due to the two phenols, carvacrol and thymol (major components of oregano essential oil) but also due to the monoterpene hydrocarbons p-cymene and γ-terpinene (Baydar et al., 2004) that occur at lower concentration (Juliano et al., 2000). According to many studies, oregano essential oil is active against a wide variety of microorganisms including Gram negative and particularly Gram positive bacteria (Sivropoulou et al., 1996). Eventhough essential oils (including oregano oil) are considered as safe (GRAS) (Lambert et al., 2001), their use is often limited by the strong odor/taste they impart to foodstuffs. For this reason the preservative effect of essential oils may be achieved by using low concentrations in combination with other preservation technologies such as low temperature (Skandamis and Nychas, 2001), low dose irradiation (Chouliara et al., 2005) and modified atmosphere packaging (Marino et al., 1999; Chouliara et al., 2006).

The objective of the present work was to study the combined effect of the O_2 absorber (Ageless®) and oregano essential oil to extend the shelf life of fresh rainbow trout fillets.

2. Materials and methods

2.1. Preparation of fish samples and storage conditions

Aquacultured freshwater rainbow trout (*O. mykiss*) weighting ca. 400 g was obtained from an aquaculture farm (GIANNETAS SA) located on river Voidomatis in North Western Greece. The fish was sacrificed by hypothermia, gutted, filleted and transferred to the laboratory (packed in polystyrene boxes containing ice) within 1 h and placed in low density polyethylene/ethylene vinylalcohol/low density polyethylene (LDPE/EVOH/LDPE) high barrier pouches, 75 μm in thickness, having an oxygen permeability of 2 $cm^3/(m^2\ d\ atm)$ at 75% relative humidity (RH), 25 °C measured using the oxygen model Oxtran 2-20 permeability tester (MOCON Minneapolis, MN).

Four lots of samples were prepared: The first lot comprised the controls (aerobic packaging). Oregano oil (Kokkinakis S.A., Athens, Greece) was pipetted to the surface of the second lot so as to obtain a final concentration equal to 0.4% v/w. The contents of the pouch were gently massaged by hand for homogenous distribution of the essential oil. Lot 3 consisted of samples in which the ZTP type O_2 absorber (Mitsubishi Gas Chemical Company, Ageless®, Japan) was added inside the package. Finally, the fourth lot consisted of samples in which both oregano oil and ZTP type O_2 absorber were added to the fish and package respectively. Pouches were heat-sealed using a BOSS model N48 vacuum sealer (BOSS, Bad Homburg, Germany) and kept at 4 °C. Sampling was carried out on day: 0, 1, 3, 5 and 7 of storage for controls samples and on day 0, 3, 6, 9, 12, 15, 18 and 21 of storage for the treated samples.

2.2. Microbiological analysis

Fish samples (25 g) were transferred aseptically into individual stomacher bags (Seward Medical, UK), containing 225 ml of sterile Buffered Peptone Water (BPW) solution (0.1%) and homogenized in a stomacher (Lab Blender 400, Seward Medical, UK) for 60 s. For each sample, appropriate serial decimal dilutions were prepared in BPW solution (0.1%). The amount of 0.1 ml of these serial dilutions of trout fillet homogenates was spread on the surface of dry media. Total viable counts (TVC) were determined using Plate Count Agar (PCA, Merck code 1.05463, Darmstadt, Germany), after incubation for 3 days at 30 °C. Pseudomonads were determined on cetrimide fusidin cephaloridine agar (Oxoid code CM 559, supplemented with SR 103, Basingstoke, UK) after incubation at 25 °C for 2 days (Mead and Adams, 1977). For members of the family Enterobacteriaceae, 1.0 ml sample was inoculated into 15 ml of molten (45 °C) violet red bile glucose agar (Oxoid code CM 485). After setting, a 10 ml overlay of molten medium was added and incubation was carried out at 37 °C for 24 h. The large colonies with purple haloes were counted. LAB were determined on de Man Rogosa Sharpe medium (Oxoid code CM 361) after incubation at 25 °C for 5 days. For H_2S-producing bacteria (including *Shewanella putrefaciens*) enumeration, a 1.0 ml sample was inoculated into 10 ml of molten (45 °C) Iron Agar (IA, Oxoid code CM 867, Basingstoke, UK). After setting, a 10 ml overlay of molten medium was added. Iron Agar plates were incubated at 20 °C and black colonies were enumerated after 2–3 days. Finally, *Clostridium* spp. were enumerated using Reinforst Clostridium Medium (RCM, Merck code 1.05410) after incubation at 35 °C for 2 days under anaerobic conditions. Anaerobic conditions were achieved by the use of Anaeropack® GENbox Jar combined with Pack-Anaero oxygen absorbers. All plates were examined visually for typical colony types and morphological characteristics associated with each growth medium. In addition, the selectivity of each medium was checked routinely by Gram staining and microscopic examination of smears prepared from randomly selected colonies from all of the media.

2.3. Physicochemical analysis

2.3.1. Determination of the headspace gas composition

On each sampling day, the headspace gas composition within each pouch was determined using a Gaspac analyzer (PBI Dansensor CheckMate 9900). Gas analysis was performed by drawing the headspace gas sample throught a syringe needle piercing a rubber septum glued on the surface of the PE/EVOH/PE pouches.

2.3.2. Drip loss

The fish sample was removed from the pouch leaving behind the drip. Drip loss (%) was measured gravimetrically by taking the weight difference of fillet of rainbow trout before and after storage under specific treatment.

2.3.3. pH determination

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

2.3.4. Lipid oxidation

The peroxide value (PV) was determined according to the official EC (2568/91) method for the measurement of the characteristics of olive oil and olive-residue oil after soxhlet extraction of the fish fat with petroleum ether for 4 h. TBA was determined according to the method of Gomes et al. (2003) as modified by Goulas and Kontominas (2007). The method is based on the spectrophotometric quantitation of the pink complex formed after reaction of one molecule of malondialdehyde (MDA), product of distillation, with two molecules of 2-thiobarbituric acid (TBA) added to the distillate.

2.3.5. Determination of total volatile basic nitrogen (TVBN)

TVBN was determined according to the method described by Pearson (1991).

2.4. Sensory evaluation

On all sampling days, samples were frozen (−20 °C) in order to be used for sensory evaluation. The attributes of cooked fish fillets were evaluated by a panel of fifty one untrained judges faculty and staff of the laboratory of Food Chemistry and Microbiology on each sampling day. Fish samples (ca. 200 g) were cooked individually in a microwave oven at high power (700 W) for 4 min including time of defrosting. Panelists were asked to evaluate taste and odor intensities of cooked samples. Along with the test samples, the panelists were presented with a freshly thawed fish sample (reference sample), stored at −20 °C throughout the experiment. Acceptability of odor and taste was estimated using a 9 point hedonic scale with 9 corresponding to the most liked sample and 1 corresponding to the least liked sample. A score of 4 was taken as the lower limit of acceptability.

2.5. Statistical analysis

Experiments were replicated twice on different occasions with different fish samples. Analysis was run in triplicate for each replicate ($n = 2 \times 3$). Microbiological data were transformed into logarithms of the number of colony forming units (cfu g^{-1}) and were subjected to analysis of variance using the software SPSS 16 for windows. Means and standard deviations were calculated and when F values were significant at the ($p < 0.05$) level.

3. Results and discussion

3.1. Microbiological changes

The changes in microbial flora of rainbow trout fillets as a function of treatment and storage time are shown in Fig. 1a–e. Initial total viable counts (Fig. 1a) for fresh rainbow fillets were ca. 2.7 log cfu/g. Most of the available literature on freshwater fish (sea bass, tilapia, rainbow trout, silver perch) reports bacterial counts of 10^2–10^6 cfu/g (Gelman et al., 2001). In this study, initial mesophilic count of 2.7 cfu/g indicates good fish quality (Dawson et al., 1995), considering the microbiological upper limit for fresh fish, proposed by ICSMF (1986). TVC reached the value of 7 log cfu/g ca. on day 4 for the air packaged samples, day 7–8 for the samples treated with oregano oil (0.4% w/v), day 9 for the samples packaged with O_2 absorber and day 12 for the packaged with O_2 absorber and containing oregano oil (0.4% w/v). The use of oregano oil resulted in a microbiological shelf life extension of 3–4 days, while the use of the O_2 absorber resulted in a shelf life extension of 5 days. The combination of the O_2 absorber plus oregano oil had a significant effect on the inhibition of TVC in rainbow trout fillets resulting in a microbiological shelf-life extension of 8 days. The combination of both the O_2 absorber and oregano oil showed an additive effect on the inhibition of the microflora in rainbow fillets. The initial mesophilic count (2.7 log cfu/g) of fresh rainbow trout found in the present study is in good agreement with results reported by Gonzalez (1999) for wild brown and farmed rainbow trout.

The Pseudomonads (Fig. 1b) were dominant in rainbow trout fillets over the entire storage period. Initial (day 0) *Pseudomonas* spp. counts were below the method detection limit (2 log cfu/g). Both oregano essential oil and O_2 absorber showed strong antimicrobial activity against the Pseudomonads. This effect may be due to the ability of carvacrol and thymol, main constituents of oregano essential oil, to attact the outer membrane of Gram negative bacteria releasing lipopolysaccharides and increasing the permeability of the cytoplasmic membrane (Burt, 2004). On the other hand the O_2 absorber substantially inhibited the growth of the aerobic Pseudomonads.

Until day 5 of storage oregano oil had no significant effect ($p > 0.05$) on the Pseudomonads. On the same day of storage, the use of O_2 absorber resulted in a reduction of 1.9 log cfu/g ($p < 0.05$) while the combination of oregano oil (0.4%) and O_2 absorber resulted in a reduction of the Pseudomonads by 2.6 log cfu/g ($p < 0.05$). As shown in Fig. 1b the use of the O_2 absorber was more effective than oregano oil. This is expected given that the Pseudomonads are strictly aerobic microorganisms and are unable to survive in the absence of oxygen.

Deans and Richie (1987) showed that thyme oil, an essential oil containing similar components as oregano oil was very effective against *Pseudomonas aeruginosa*, in a study where the inhibitory properties of ten plant essential oils were tested using an agar diffusion technique. Skandamis et al. (2002) reported that the Pseudomonads were the most resistant bacteria group to oregano oil. Ouattara et al. (1997), reported low inhibition effects of both oregano and thyme oils on a number of bacteria such as *Pseudomonas fluorescens*, *Brochothrix thermosphacta* and *Lactobacillus sakei*. The above results regarding the population of *Pseudomonas* spp, are in agreement with those of Mohan et al. (2008) who reported a reduction of psychrotrophs by ca. 2 log cfu/g for O_2 scavenger packs vs. control air packs for catfish during a 20 day storage period. Also Pantazi et al. (2008), reported that the Pseudomonads exceeded 7 log cfu/g on day 6 of storage (for aerobically packaged swordfish samples) and on day 11–12 (for vacuum packaged samples).

H_2S producing bacteria including *S. putrefaciens* are also specific spoilage microorganisms for fish. *S. putrefaciens* produces very intense and unpleasant off-odors and reduces TMAO to TMA, producing H_2S (Sivertsvik et al., 2002). On day 5 of storage, the H_2S-producing bacterial counts (including *S. putrefaciens*) reached 5.7 log cfu/g for aerobically-packaged samples (Fig. 1c). On the same day H_2S producing bacterial counts were reduced by 1.4 log cfu/g (oregano oil 0.4%) ($p < 0.05$), 1.5 log cfu/g (O_2 absorber) and ca. 2.5 log cfu/g (oregano oil 0.4% and O_2 absorber). H_2S producing bacteria grew sufficiently in the presence of the O_2 absorber and reached the value of 7.5 log cfu/g on day 18 of storage.

Enterobacteriaceae, a hygiene indicator, were also part of the microflora of fresh rainbow trout. This is in agreement with the findings of Papadopoulos et al. (2003) regarding the microflora of Mediterranean fish. On a given sampling day (day 5) Enterobacteriaceae counts (Fig. 1d) reached the value of 7.5 log cfu/g for aerobically-packaged samples. On the same day Enterobacteriaceae counts were reduced by 2.5 log cfu/g in the presence of oregano oil 0.4% ($p < 0.05$) by 2.9 log cfu/g in the presence of the O_2 absorber and by ca. 4.0 log cfu/g in the presence of oregano oil 0.4% plus the O_2 absorber. The contribution of Enterobacteriaceae to the microflora of fish and its potential spoilage must be taken into consideration in case of polluted waters or delay in chilling after catch.

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 (Fig. 1e). The initial LAB count was ca. 2.2 log cfu/g (on day 0) and reached a count of 6.0 log cfu/g on day 5 of storage for the air packaged samples. On the same day, the use of O_2 absorber and oregano essential oil resulted in a reduction of LAB by 0.3 and 2.3 log cfu/g, respectively, while the combination of oxygen absorber and oregano essential oil resulted in a reduction in LAB counts by almost 2.6 log cfu/g ($p < 0.05$). These findings are in general agreement with the results of Zaika et al. (1983), who reported a reduction of LAB by 2 log cfu/g in a pure culture after the addition of oregano oil at a concentration of 4 g/l. They are also in agreement with those of Chouliara and Kontominas. (2006) who reported

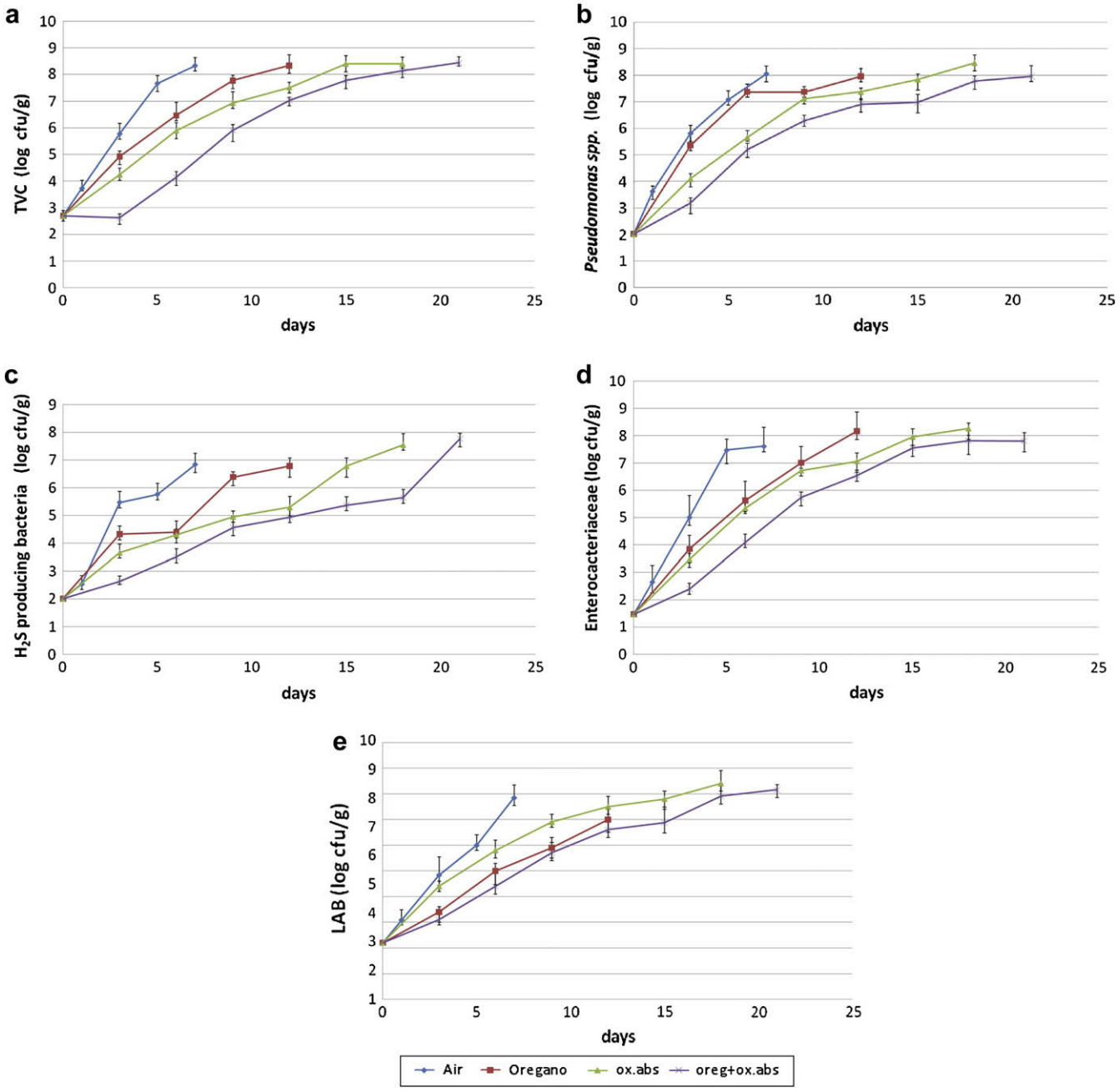

Fig. 1. a: Combined effect of oxygen absorber and oregano essential oil on TVC in rainbow trout fillets stored at 4 °C, b: Combined effect of oxygen absorber and oregano essential oil on *Pseudomonas spp*. counts in rainbow trout fillets stored at 4 °C, c: Combined effect of oxygen absorber and oregano essential oil on H_2S producing bacterial population in rainbow trout fillets stored at 4 °C, d: Combined effect of oxygen absorber and oregano essential oil on Enterobacteriaceae population in rainbow trout fillets stored at 4 °C, e: Combined effect of oxygen absorber and oregano essential oil on LAB population in rainbow trout fillets stored at 4 °C.

a reduction of 1.1 log cfu/g in LAB for chopped chicken meat after 6 days of storage with the addition of 0.1% oregano essential oil.

Clostridium spp. counts remained below the method detection limit (1 log cfu/g) throughout storage for all samples.

3.2. Sensory analysis

The results of odor and taste evaluation of rainbow trout fillets are presented in Fig. 2a and b, respectively. The score for both odor and taste decreased significantly ($p < 0.05$) over storage. Both odor and taste proved to be equally sensitive attributes of rainbow trout fillet quality. Based both on taste and odor, shelf life was 4 days for the air packaged samples, 7–8 days for the air packaged samples plus oregano essential oil, 13–14 days for samples containing the O_2 absorber and 17 days for samples containing the O_2 absorber plus oregano oil.

Agreement between microbiological and sensory data was excellent regarding the controls as well as samples containing oregano oil but poor regarding samples containing the oxygen absorber. Poor correlation between microbiological and sensory data has been documented previously by several workers (Chytiri et al., 2004; Chouliara et al., 2005) and may be related to the fact that specific spoilage microorganisms and not TVC are responsible

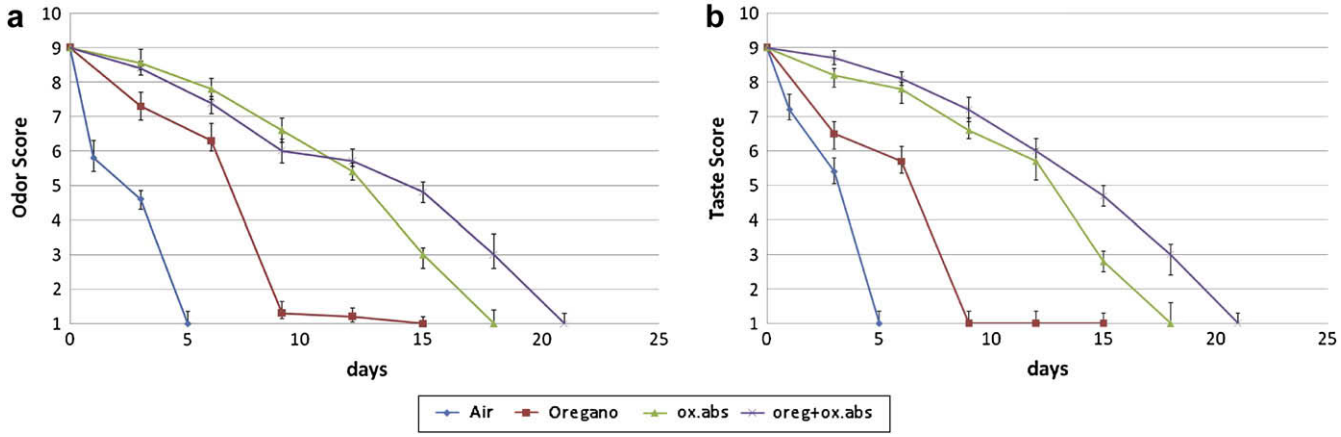

Fig. 2. a: Combined effect of oxygen absorber and oregano essential oil on odor of rainbow trout fillets stored at 4 °C, b: Combined effect of oxygen absorber and oregano essential oil on taste of rainbow trout fillets stored at 4 °C.

for the spoilage of fish. Mohan et al. (2008) reported a shelf life extension for catfish steaks of 10 days in O_2 scavenger packs as compared to control air packs. Giatrakou et al. (2008) similarly reported a shelf life extension of swordfish of 5–6 days in case of addition of oregano essential oil at a concentration of 0.1%, v/w.

3.3. Physicochemical changes

3.3.1. Head space gas composition

The oxygen absorber reduced oxygen concentration to less than 0.01% and maintained these levels throughout the entire storage period (data not shown). Such a reduction was also observed in catfish (Mohan et al., 2008), and sea bream (Gonçalves et al., 2004). CO_2 concentration in packages increased with storage time. After 21 days of storage CO_2 concentration in the headspace reached 56.9 in samples with the O_2 absorber and 46.9% in samples packaged with the oxygen absorber in combination with oregano essential oil. CO_2 build up is a result of increased microbial activity during storage of rainbow trout fillets. Mohan et al. (2008) and Gonçalves et al. (2004) also reported similar results of increase in CO_2 concentration during storage of catfish and sea bream in the presence of oxygen absorbers.

3.3.2. Drip loss

The drip loss ranged between 7% (on day 4) for control samples and 11–12% (on day 17–18) for samples containing the O_2 absorbers and oregano essential oil at the point of sensory rejection (Fig. 3a). Similar results were also reported by Mohan et al. (2008) for catfish.

High drip loss results to a lower quality product due to 1) poorer texture resulting in the product after cooking and 2) more rapid growth of microorganisms using drip liquid as growth medium.

3.3.3. pH

The initial pH (data not shown) of rainbow trout fillets was 6.65 which is in agreement with that of Gimenez et al. (2002) and Chytiri et al. (2004). In the case of air packaged samples pH decreased from 6.65 (day 0) to 6.22 at the time of sensory rejection (day 4). Respective pH values were 6.13 for samples containing oregano essential oil (day 7–8 of storage) 6.20 for samples containing the O_2 absorber (day 13–14 storage) and 6.41 for samples containing the O_2 absorber plus oregano oil. In the case of treated trout fillets there was a trend of increasing pH values (i.e. 6.86) during the later stages of storage. Such a trend is owed to the production of alkaline compounds such as ammonia due to protein decomposition (Mohan et al., 2008).

3.3.4. TVBN

Fig. 3b shows TVBN values (mg N_2/100 g trout) for trout fillets as a function of treatment and storage time. Initial (day 0) TVBN value in rainbow trout fillets was 10.6 mg N_2/100 g in agreement with literature data (Gimenez et al., 2002). At the time of sensory rejection TVBN values were ca. 20 mg N_2/100 g for air packaged samples, 25 mg N_2/100 g for samples containing oregano essential oil, 42.5 mg N_2/100 g for samples containing O_2 absorber and 54.6 mg N_2/100 g for samples containing O_2 absorber plus oregano essential oil. Generally, such values only reflect advanced spoilage, and are not considered very reliable for measuring the deterioration of certain fish species (Castro et al., 2006; Tejada et al., 2007; Özogul et al., 2007). Castro et al. (2006) did not observe an increase in volatile bases until after 20–22 days of storage, when the fish was already considered unfit for human consumption. On the contrary Chytiri et al. (2004) reported TVBN values of 18.31 mg N_2/100 g for filleted trout samples stored in ice at the time of sensory rejection (10–12 days). Based on the above it may be concluded that TVBN cannot always be used as a quality index of fish as shown in the present study.

3.3.5. Lipid oxidation

Rainbow trout is rich in monounsaturated (50%) and polyunsaturated (26%) fatty acids (Kotakowska et al., 2006) and therefore very sensitive to lipid oxidation which limits its shelf life. In the present study primary (PV) and secondary (TBA) oxidation products were determined as indicators of the degree of lipid oxidation. Peroxide values are given in Fig. 3c. The initial peroxide value of rainbow trout fillet was 11.4 meq O_2/kg rainbow fish oil. Similar PV values have been reported from Hansen (1963) for rainbow trout oil (12 meq O_2/kg rainbow trout oil). Respective PV values for air packaged samples plus oregano oil were 27 meq O_2/kg oil, for samples with O_2 absorbers 14 meq O2/kg oil and for samples with O_2 absorbers plus oregano oil 12 meq O2/kg oil. It is noteworthy to mention that the use of O_2 absorbers retained PV values $\leq$ 20 meq O_2/kg oil even after 21 days of storage which substantially exceeds the sensory shelf life of trout fillets.

As shown in Table 1 correlation between microbiological and sensory attributes, between physicochemical and sensory attributes and between microbiological and physicochemical attributes was generally poor. Exceptions to this general statement were the

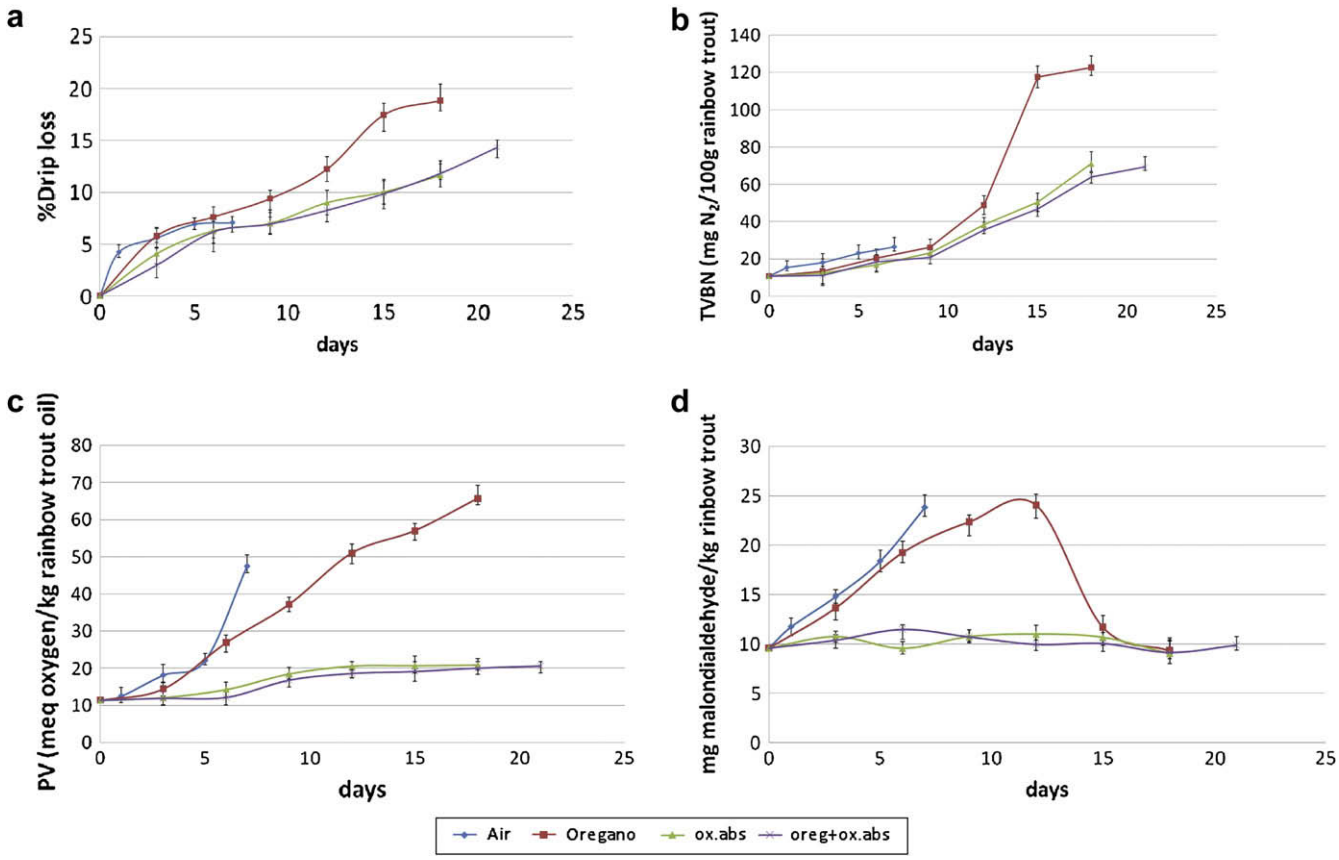

Fig. 3. a: Combined effect of oxygen absorber and oregano essential oil on drip loss in rainbow trout fillets stored at 4 °C, b: Combined effect of oxygen absorber and oregano essential oil on Total volatile basic nitrogen in rainbow trout fillets stored at 4 °C, c: Combined effect of oxygen absorber and oregano essential oil on Peroxide value of rainbow trout fillets stored at 4 °C, d: Combined effect of oxygen absorber and oregano essential oil on malondialdehyde content of rainbow trout fillets stored at 4 °C.

positive correlation between H_2S-producing bacterial count and odor, between PV and TBA and between drip loss and taste.

According to the literature a PV value of ca.20 meq O2/kg oil is considered as the upper limit for foodstuffs (Özogul et al., 2005, 2006). Similar results for PV were obtained by Mohan et al. (2008) for sea bream. In this case the major protective effect is owed to the use of the oxygen absorber and to a smaller degree to the antioxidant effect of oregano essential oil. Secondary lipid oxidation products include malondialdehyde oxidation product of linoleic acid. The initial MDA value of rainbow trout fillets was 9.6 mg MDA/kg rainbow trout (Fig. 3d). Similar MDA values have been reported by Chytiri et al. (2004) for the same substrate. MDA values increased sharply in the case of air packaged samples and samples containing oregano oil. In samples containing the oxygen absorber the increase in MDA content was statistically insignificant ($p > 0.05$). This finding is very important as secondary oxidation products cause unpleasant odors in fish muscle. According to Auburg (1993), TBA values may not reflect the actual rate of lipid

Table 1
Correlation among microbiological, physicochemical and sensory attributes of rainbow trout fillets.

	TVC	*Pseudomonas* spp.	LAB	H_2S-producing bacteria	Enterobacteriaceae	Odor	Taste	pH	PV	TBA	TVBN	Drip loss
TVC												
Pseudomonas spp.	0.700											
LAB	0.827	0.096										
H_2S-producing bacteria	0.008	0.803	0.612									
Enterobacteriaceae	0.266	0.913	0.742	0.084								
Odor	0.140	0.713	0.272	0.038	0.312							
Taste	0.538	0.612	0.803	0.205	0.173	0.099						
pH	0.742	0.106	0.493	0.738	0.827	0.866	0.912					
PV	0.354	0.592	0.364	0.381	0.125	0.866	0.803	0.674				
TBA	0.397	0.461	0.389	0.288	0.072	0.781	0.257	0.870	0.024			
TVBN	0.072	0.288	0.700	0.103	0.208	0.417	0.658	0.425	0.257	0.266		
Drip loss	0.397	0.354	0.461	0.461	0.704	0.165	0.007	0.499	0.321	0.623	0.544	

Correlation is significant at level 0.05.

oxidation since malondialdehyde can interact with other components of fish muscle. Such components may be amines, nucleosides and nucleic acids, proteins, amino acids of phospholipids, such an interaction varying greatly with fish species. This may explain the decrease in TBA values after day 12 of storage in samples containing oregano essential oil. Such an MDA trend has also been reported by Goulas and Kontominas (2007) and Chouliara et al. (2004) for sea bream and Papadopoulos et al. (2003) for gutted sea bass.

4. Conclusion

The present study showed that the combination of the Ageless® oxygen absorber and oregano essential oil at a concentration of 0.4% v/w was very effective in extending the shelf life of fresh rainbow trout fillets to 17 days, whereas samples packaged aerobically had a shelf life of only 4 days. In turn, a shelf life of 7–8 days was obtained for aerobically-packaged samples with the addition of oregano oil and 13–14 days for samples containing the oxygen absorber. Present results are based primarily on sensory evaluation and secondarily on microbiological analysis. Chemical indices' data were not in good agreement with sensory and microbiolgical data and thus cannot be used as quality indices of rainbow trout fillets.

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