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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.

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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).

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