Journal of Food Safety

Journal of Food Safety ISSN 1745-4565

ANTIMICROBIAL EFFECTIVENESS OF GELATIN–ALGINATE FILM CONTAINING OREGANO ESSENTIAL OIL FOR FISH PRESERVATION

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Received for Publication December 10, 2014
Accepted for Publication March 21, 2015

doi: 10.1111/jfs.12198

ABSTRACT

This study aimed to evaluate the effect of gelatin–alginate film containing 1.5% oregano essential oil (OEO) on the shelf life of rainbow trout (*Oncorhynchus mykiss*) slices during refrigerated storage over a period of 15 days. The blend film was prepared at 75% fish gelatin to 25% sodium alginate ratio and OEO was added to the film formulation. All the treatments (the control and wrapped slices) were analyzed periodically in terms of microbiological factors (total viable count, psychrotrophic count and spoilage microorganism, such as lactic acid bacteria, *Pseudomonas* spp. and Enterobacteriaceae). In addition, the samples were analyzed in terms of total volatile base nitrogen (TVB-N) and pH. Use of the OEO–blend film delayed bacterial growth throughout 15 days of storage compared with the control and slices treated with blend films without OEO ($P < 0.05$). The lowest TVB-N and pH levels were 59.98 and 6.75, respectively, in OEO film at the end of the storage. Therefore, this study showed that OEO–blend film was an effective antimicrobial suitable for the potential food packaging applications.

PRACTICAL APPLICATIONS

Development of edible films incorporated with essential oils is an attractive option for improving packaging food. In this study, the effect of gelatin–alginate edible film containing oregano essential oil (OEO) on spoilage bacteria was investigated. Strong antimicrobial effect of OEO and restricted access of oxygen by films make gelatin–alginate film incorporated with OEO suitable for fish packaging.

INTRODUCTION

Fresh fish is a highly perishable product due to its biological composition (Arashisar *et al.* 2004; Ojagh *et al.* 2010; López de Lacey *et al.* 2013). The major cause of food spoilage is microbial growth and metabolism (Gram and Dalgaard 2002). Thus, many strategies have been developed to inhibit spoilage and pathogenic microorganisms (Gómez-Estaca *et al.* 2010), one of which is the incorporation of antimicrobial compounds into edible films and coatings to prevent surface growth in food where a large portion of spoilage and contamination occurs (Gyawali and Ibrahim 2014). Edible film can be prepared from natural polymers such as proteins, polysaccharides and lipids (Emiroğlu *et al.* 2010; Tajkarimi *et al.* 2010; Iturriaga *et al.* 2012; Wu *et al.* 2014). As a typical protein film-based material, gelatin is used due to its abundance, acceptability, low manufacturing cost and great film-forming properties (Bae *et al.* 2009). However, the main drawbacks of gelatin application are its poor mechanical properties and moisture sensitivity. Blending gelatin with polysaccharides improves its film formation (Wu *et al.* 2014). Alginate has good potential for film and coating formation due to its colloidal properties, including strength, thickness, emulsion stability, and gel and film formation (Rhim 2004; Maizura *et al.* 2007). Therefore, it can be combined with gelatin in order to provide a better film.

Essential oils, as natural antimicrobials, can be incorporated into film formulation to increase shelf life by delaying spoilage (Zivanovic *et al.* 2005; Gómez-Estaca *et al.* 2010). In order to directly achieve effective antimicrobial activity of essential oils in food, its high concentrations should be used (Emiroğlu *et al.* 2010; Royo *et al.* 2010). The advantage

of incorporating essential oil into the film is that it could attain the desired goal with lower oil concentrations, thus limiting unwanted flavors and odors to the food (Zinoviadou *et al.* 2009; Gómez-Estaca *et al.* 2010; Royo *et al.* 2010; Salgado *et al.* 2013; Wu *et al.* 2014). Moreover, its effect remains for a longer period (Benavides *et al.* 2012). Oregano essential oil (OEO) has antimicrobial and antioxidant properties and can be frequently used in food preservation (Chouliara *et al.* 2007). The antimicrobial activity of OEO is due to carvacrol and thymol as its major compounds (Chouliara *et al.* 2007; Mexis *et al.* 2009).

Moreover, other researchers have also shown that the incorporation of essential oils into the film matrix improves food safety and extends the shelf life of fish (Jeon *et al.* 2002; Gómez-Estaca *et al.* 2010; Ahmad *et al.* 2012; Jouki *et al.* 2014; Wu *et al.* 2014).

Nevertheless, few studies have been conducted about the effect of blend films containing essential oils on reducing spoilage bacteria. Therefore, this investigation aimed to study the combined antimicrobial effect of OEO and gelatin–alginate film on the shelf life extension of fresh rainbow trout slices stored at 4C.

MATERIALS AND METHODS

Preparation of OEO–Blend Films and Treated Slices

Cold water fish skin gelatin (Sigma Chemical Co., Cat. No. G7041, solid form, bioreagent) and sodium alginate (High Science Limited Partnership Co., Bangkok, Thailand) solutions were prepared separately. About 3 g of gelatin and 1.5 g of alginate were dispersed in 100 mL of distilled water and film solutions were prepared using the methods proposed by Ahmad *et al.* (2012) and Alboofetileh *et al.* (2013), respectively. Film solutions were mixed in 75% gelatin and 25% alginate ratio, and glycerol (0.25 g/g of biopolymer) was added as a plasticizer. Oregano (*Origanum vulgare*) essential oil (OEO) (New Direction, Hampshire, U.K.), which had been previously mixed with Tween 80 (Merck Co., Darmstadt, Germany) as an emulsifier (0.20 g/g of essential oil) to assist dispersion, was then added to the film solution to reach the final concentration of 1.5% w/v. The film solutions were homogenized at 13,500 rpm for 4 min at room temperature using a homogenizer (Wiggen Hauser, D-500, Berlin, Germany). The film-forming solutions were degassed under vacuum for 10 min. The filmogenic solution was cast onto an 8-cm diameter Petri dish and dried at 30C in an oven for approximately 48 h. Dried film samples were aseptically peeled off and used for wrapping the rainbow trout slices.

Fresh rainbow trout (*Oncorhynchus mykiss*) was obtained from an aquaculture farm located in Chamestan, Mazandaran Province, Iran, and transported in ice in insulated polystyrene boxes and delivered to the laboratory within 20 min. Immediately after the delivery, the whole fish was filleted, sliced to the thickness of 1.5 cm, and for each slice, films were placed on both sides. The samples were placed in polyethylene bags and stored at refrigerated temperature (4 ± 0.5C) for 15 days. The treatments were as follows: control samples, slices covered with film and slices covered with OEO film. Sampling was carried out at predetermined time intervals, namely 0, 3, 6, 9, 12 and 15 days of storage.

Characterization of OEO

For determination of OEO, it was analyzed on an Agilent 6890 gas chromatography (GS) connected to a Hewlett-Packard, model 5973, mass spectrometric (MS) detector (Agilent Technologies, Wilmington, DE). A capillary column DB-5MS (60 m length $\times$ 0.320 mm internal diameter and 1-μm film thickness) was used for the separation of individual components of the OEO. The chemical composition of EO was determined according to the method described by Karabagias *et al.* (2011). The oven temperature was programmed at 100C for 5 min, increased by 10C/min to 130C, increased by 7C/min to 270 and held to 270C for 3 min. Helium was used as the carrier gas at a flow rate of 1.5 mL/min. Further, 0.1% solution of EO was prepared in hexane and 1 μL of this solution was injected. The injector was operated in split mode (20:1 split ratio) at a temperature of 270C. The mass spectrometer was operated under the following conditions: scan range: 30–330; source temperature: 230C; quadrupole temperature: 150C; electron impact (EI) ionization: 70 eV. Identification of compounds was achieved by comparing the mass spectra of the recorded chromatographic peaks with the Wiley 275 MS database.

Microbiological Analyses

The microbial counts on the slices were determined by homogenizing 10 g of the samples without films in 90 mL of 0.85% NaCl. Appropriate dilutions were prepared from the homogenized samples in the same dilutions for determining the following microorganisms.

Total Viable Count (TVC) and Psychrotrophic Bacterial Count. TVC and psychrophilic bacterial count were determined by the pure plate method using plate count agar (QUELAB, Montreal, Canada), which was followed by incubation at 30C for 72 h and 7C for 10 days, respectively.

***Pseudomonas* Spp.** *Pseudomonas* spp. were determined on Pseudomonas agar (QUELAB) with added CFC (cetrimide, fucidin, cephalosporin) supplement after incubation at 25C for 48 h.

Enterobacteriaceae Count. For determining enterobacterial count, 1.0 mL of diluted sample was placed in sterile Petri plates and covered with a layer of molten violet red bile dextrose agar (QUELAB). After being placed at room temperature, 10 mL overlay of the same medium was added and incubated at 30C for 48 h.

Lactic Acid Bacteria (LAB) Count. LAB were enumerated on double-layered palates of de Man Rogosa Sharpe agar (QUELAB) incubated at 30C for 72 h.

Four replicates were made for each test sample and the least three appropriate dilutions were used for each replicate. The results were expressed as the log of the number of colony-forming units per gram (log cfu/g) of muscle.

Determining Total Volatile Basic Nitrogen (TVB-N) Content

TVB-N value was estimated by the microdiffusion method contents described by Goulas and Kontominas (2005). The microdiffusion method was determined by the distillation of the fish sample after adding magnesium oxide (MgO). The distillate was collected in a flask containing boric acid aqueous solution and methyl red as an indicator. Afterward, the boric acid solution was titrated with a sulfuric acid solution. The TVB-N value (mg N/100 g of fish) was determined according to the consumption of sulfuric acid. Determinations were carried out at least in triplicate.

pH Measurement

The pH value of the slice sample was recorded using a standard calibrated pH meter. About 5 g of fish slice was homogenized with 45 mL of distilled water. After 5 min, pH measurement was performed on days 0, 3, 6, 9, 12 and 15 of the refrigerated storage. The experiments were repeated at least in triplicate.

Statistical Analyses

The results were reported as mean values ± standard deviation (SD). The data were subjected to analysis of variance (ANOVA) using SPSS software for Windows (version 17.0, SPSS Inc., Chicago, IL), and Duncan's multiple range test was employed to detect significant ($P < 0.05$) differences.

RESULTS

Chemical Composition of OEO

The major components of OEO are listed in Table 1. Carvacrol was the predominant compound (81.85%), followed by *p*-cymene (4.01%), thymol (3.30%) and γ-terpinene (2.26%). The result of the chemical composition of OEO is indicative of a high level of antimicrobial components (carvacrol, thymol, *p*-cymene and γ-terpinene).

TABLE 1. COMPOSITION OF OREGANO ESSENTIAL OIL[a]

Compounds	% composition
α-Pinene	0.32
β-Pinene	0.64
p-Cymene	4.07
1,8-Cineole	1.24
γ-Terpinene	2.26
Linalool	1.52
Camphene	0.69
endo-Borneol	0.75
4-Terpinene	0.41
α-Terpinene	0.36
Thymol	3.30
Carvacrol	81.85
B-myrcene	0.42
Caryophyllene	1.44
α-Terpineol	0.36

[a] Expressed as percentage of the total peak area of the chromatograms.

Microbiological Analysis

The change in the TVC of the fish slices during storage time is shown in Fig. 1. The initial value of TVC (day 0) for the samples was 2.5 log cfu/g. The upper acceptable microbiological limit recommended by the International Commission on Microbiological Specifications for Foods (ICMSF)

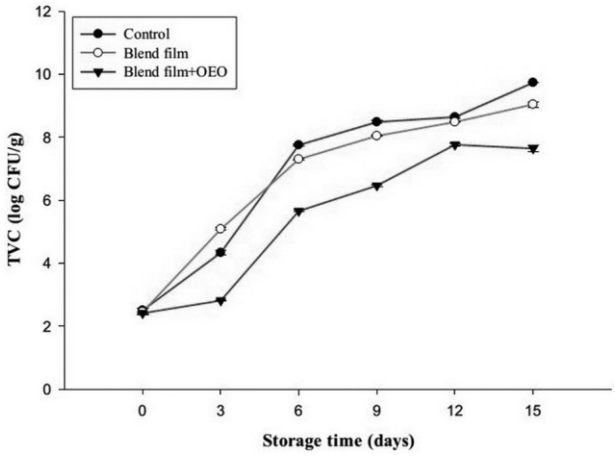

FIG. 1. CHANGES IN THE TOTAL VIABLE COUNT (TVC) OF RAINBOW TROUT SLICES WRAPPED WITHOUT AND WITH FILMS (OREGANO ESSENTIAL OIL [OEO]–BLEND FILM OR BLEND FILM) DURING STORAGE TIME AT 4C
Error bars show SD.

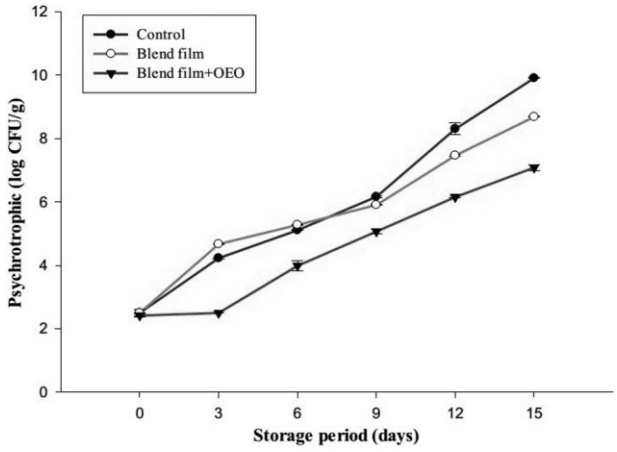

FIG. 2. CHANGES IN PSYCHROTROPHIC COUNTS OF RAINBOW TROUT SLICES WRAPPED WITHOUT AND WITH FILMS (OREGANO ESSENTIAL OIL [OEO]–BLEND FILM OR BLEND FILM) DURING STORAGE TIME AT 4C
Error bars show SD.

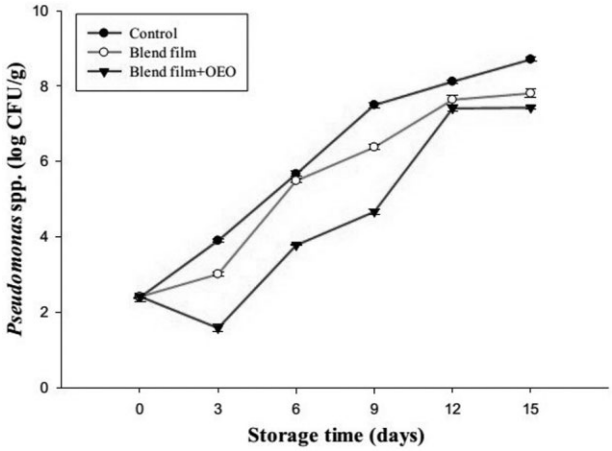

FIG. 3. CHANGES IN *PSEUDOMONAS* SPP. COUNTS OF RAINBOW TROUT SLICES WRAPPED WITHOUT AND WITH FILMS (OREGANO ESSENTIAL OIL [OEO]–BLEND FILM OR BLEND FILM) DURING STORAGE TIME AT 4C
Error bars show SD.

for TVC was 7 log cfu/g for freshwater and marine fish (ICMSF 2002). TVC reached the value of 7 log cfu/g on day 5 for the control samples, day 6 for the samples treated with blend film and day 11 for the samples treated with OEO–blend film. Further, 1.5% OEO incorporated blend film reduced the growth of TVC and reached approximately 2 log reduction on the fish slice as compared with the control sample ($P < 0.05$) on day 15.

The results of psychrotrophic bacterial count (Fig. 2) showed that the initial counts increased from 2.5 log cfu/g to 9.91, 8.69 and 7.09 log cfu/g for the control samples, samples treated with the blend film and samples wrapped with OEO-enriched film at the end of the storage period, respectively.

Growth of *Pseudomonas* spp. on the rainbow trout slices and the effect of blend films with and without OEO are presented in Fig. 3. Initial counts on Pseudomonas agar for *Pseudomonas* spp. demonstrated 2.38 log cfu/g (day 0). Growth pattern of *Pseudomonas* spp. demonstrated the same behavior as TVC and psychrotrophic, and the control was at the highest level on day 15 (8.71 log cfu/g). It was followed by the sample treated with the blend film (7.81 log cfu/g), while the lowest count was detected in the sample wrapped with OEO–blend film. The number of *Pseudomonas* spp. was reduced by the effect of the films. These reductions in *Pseudomonas* spp. count were significant ($P < 0.05$), showing reductions of 0.9 and 1.37 logarithmic cycles for the samples treated with blend film and OEO–blend film, respectively.

Enterobacteriaceae counts showed increases in all treatments during storage time ($P < 0.05$) (Fig. 4). The samples wrapped with OEO–blend film had significantly lower Enterobacteriaceae count of about 2.17 log cfu/g than the control on day 15 of storage.

Changes in the LAB counts of the rainbow trout slices treated with blend film with and without OEO during the storage are shown in Fig. 5. Initial (day 0) LAB count was 3 log cfu/g, which gradually increased during the storage time and reached the final populations of 6.57 log cfu/g

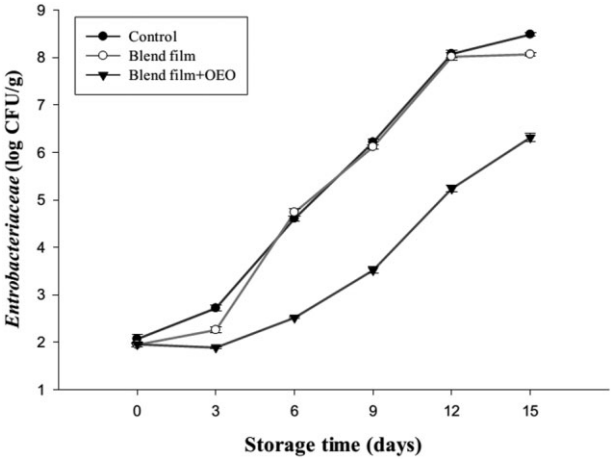

FIG. 4. CHANGES IN ENTEROBACTERIACEAE OF RAINBOW TROUT SLICES WRAPPED WITHOUT AND WITH FILMS (OREGANO ESSENTIAL OIL [OEO]–BLEND FILM OR BLEND FILM) DURING STORAGE TIME AT 4C
Error bars show SD.

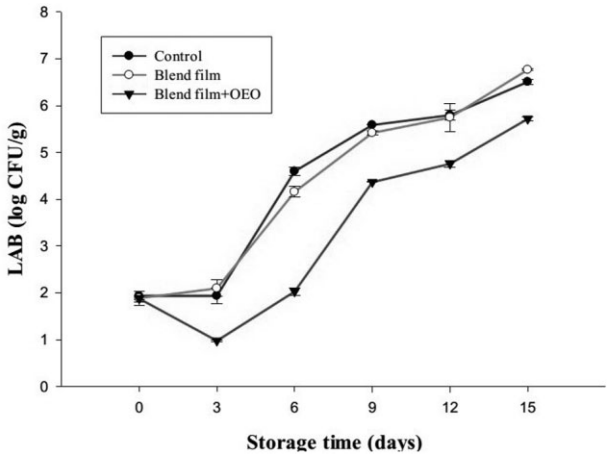

FIG. 5. CHANGES IN LACTIC ACID BACTERIA LAB COUNTS OF RAINBOW TROUT SLICES WRAPPED WITHOUT AND WITH FILMS (OREGANO ESSENTIAL OIL [OEO]–BLEND FILM OR BLEND FILM) DURING STORAGE TIME AT 4C
Error bars show SD.

(control sample), 6.77 log cfu/g (sample treated with the blend film) and 5.72 log cfu/g (sample wrapped with OEO–blend film).

TVB-N

Changes in the TVB-N value for all the treatments during the storage time are presented in Fig. 6. The initial TVB-N value in the rainbow trout slices was 10.37 mg/100 g muscle

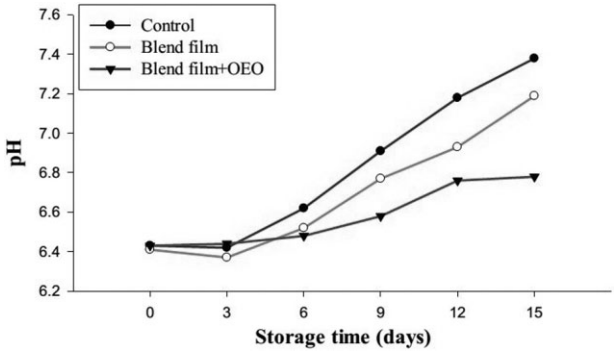

FIG. 7. CHANGES IN pH VALUES OF RAINBOW TROUT SLICES WRAPPED WITHOUT AND WITH FILMS (OREGANO ESSENTIAL OIL [OEO]–BLEND FILM OR BLEND FILM) DURING STORAGE TIME AT 4C

and increased gradually throughout the storage time in all the samples. TVB-N values of both unwrapped and wrapped samples increased significantly ($P < 0.05$) with storage time. The control samples and samples wrapped with blend film were accepted up to day 9. On the contrary, use of the OEO–blend film significantly increased the acceptance of the slices ($P < 0.05$) compared with the other samples.

pH Measurement

The effects of the treatments on the pH values during storage periods at 4C are shown in Fig. 7. Initially, the pH of the rainbow trout was 6.4. The pH value gradually increased

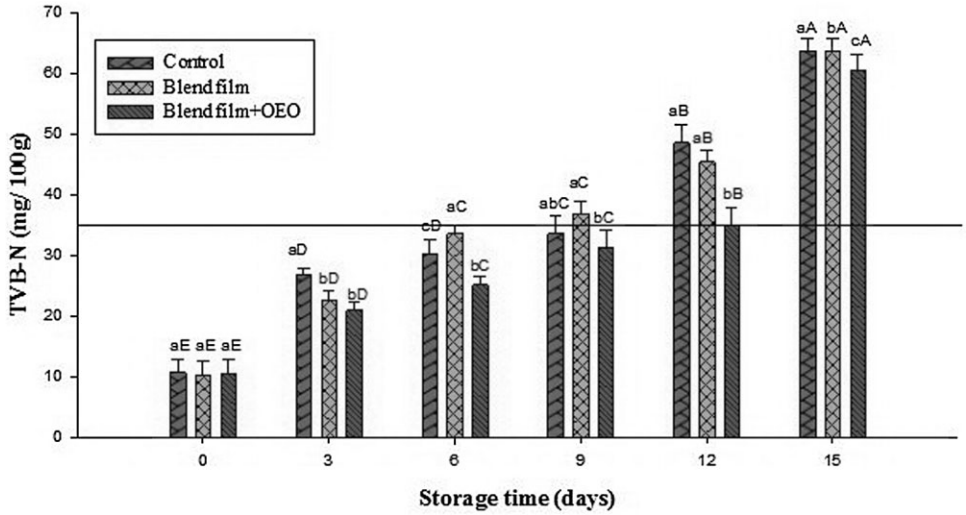

FIG. 6. CHANGES IN TOTAL VOLATILE BASIC NITROGEN (TVB-N) VALUES OF RAINBOW TROUT SLICES WRAPPED WITHOUT AND WITH FILMS (OREGANO ESSENTIAL OIL [OEO]–BLEND FILM OR BLEND FILM) DURING STORAGE TIME AT 4C
Different lowercase letters (a, b, c) represent significant differences between sampling dates for a given group. Different capital letters (A, B, C) represent significant differences between groups on different sampling dates.

in all the treatments during the storage time; in OEO–blend film, this increase was slower compared with the control samples and the slices treated with blend film.

DISCUSSION

In fresh fish, specific spoilage organisms (SSOs) constitute only a minor part of the total microflora. During storage period, SSOs grow faster than the remaining microflora, produce metabolites responsible for off-flavors or off-odors, and consequently cause consumer food rejection (Dalgaard 2003). *Pseudomonas* spp. and LAB are generally predominant in spoiled fish flora, while different gram-negative bacteria, including Enterobacteriaceae, are frequently present (Gram and Huss 1996). SSO level is correlated with the shelf life and total bacterial level of fresh fish (Dalgaard 2003). Below, the antimicrobial effect of the OEO–blend film on the spoilage bacteria of rainbow trout slices stored in the refrigeration is discussed.

In the present study, the initial TVC was low. Similarly, low initial TVC count (between 2.5 and 3.08 log cfu/g) has been reported for fresh rainbow trout fillet (Lyhs *et al.* 2001; Mexis *et al.* 2009; Pyrgotou *et al.* 2010; Nowzari *et al.* 2013). Zinoviadou *et al.* (2009) reported that the use of whey protein isolate films containing 1.5% OEO resulted in 3.3 log cfu/g reduction of the TVC population on day 8 of the refrigerated storage on fresh beef cuts as compared with the control. Such a significant reduction in the aforementioned study might be due to the differences in the applied meaty material (Emiroğlu *et al.* 2010).

The gram-negative psychrotrophic bacteria are a major group of microorganisms that are responsible for the aerobic spoilage of fresh fish stored at refrigerated temperature (Gram and Huss 1996; Ibrahim Sallam 2007) and use of the OEO–blend film led to a significant reduction of psychrotrophic bacteria ($P < 0.05$).

Growth of *Pseudomonas* spp., as more important SSOs in aerobically stored fresh fish, in the samples treated with the films is lower than that of the control samples. In other studies, use of whey protein isolate film containing 1.5% OEO and oregano significantly ($P < 0.05$) reduced *Pseudomonas* spp. population at beef cuts and rainbow trout fillet, respectively, by the end of storage time, compared with the control samples (Mexis *et al.* 2009; Zinoviadou *et al.* 2009; Pyrgotou *et al.* 2010).

The low initial value of TVC (day 0) indicated good processing conditions. Also, the low initial count of Enterobacteriaceae as a hygiene indicator (about 2 log cfu/g) demonstrated good hygiene of the water environment. Spoilage potential of Enterobacteriaceae must be considered, especially in the case of polluted water or delay in the chilling of fish after capture (Ibrahim Sallam 2007).

As can be seen, no significant difference was observed ($P > 0.05$) between LAB and Enterobacteriaceae population growth in the control and samples treated with the blend film. Regarding the LAB counts, this finding was in agreement with the results reported by Jouki *et al.* (2014), who reported the use of quince seed mucilage film without EO. Also, use of the blend film containing OEO reduced the LAB ($P < 0.05$) and inhibited the growth of Enterobacteriaceae on the first three days of storage time. In other studies, similar results have been reported, such as using OEO on trout fillet (Mexis *et al.* 2009; Frangos *et al.* 2010; Pyrgotou *et al.* 2010) and OEO on *Octopus vulgaris* (Atrea *et al.* 2009).

Gram-negative organisms are more resistant to the action of antimicrobials because they possess an outer membrane surrounding the cell wall, which restricts the diffusion of hydrophobic compounds through its lipopolysaccharide covering (Burt 2004). In the present study, the least inhibitory effect of the films containing essential oil was observed in the LAB and followed by *Pseudomonas* spp. The LAB population is of little importance for sensory changes during the storage of fish products (Gram and Huss 1996), but it is a part of the natural microflora of rainbow trout that can grow under both anaerobic and aerobic conditions (Mexis *et al.* 2009). LAB, among the gram-positive bacteria, is the most resistant one to the antimicrobial action of EOs (Kostaki *et al.* 2009). The limited action of EOs has been attributed to the high tolerance of LAB against the action of EOs. It could be related to their better ability to deal with the conditions of osmotic stress and their more effective response to K^+ efflux caused by many EOs (Frangos *et al.* 2010; Jouki *et al.* 2014). Although, among the gram-negative bacteria, Pseudomonads appear to be the least sensitive to the action of EOs (Burt 2004) and the film contains OEO as well as a reduction in Pseudomonas growth compared with controls.

In the present study, OEO–blend films were demonstrated to be effective for delaying microbial growth. The antimicrobial activity of EOs is related to their chemical composition, mainly the phenolic components (Gómez-Estaca *et al.* 2010). Gas chromatography–mass spectrography (GC-MS) analysis of OEO showed that it is rich in phenolic monoterpenoids, mainly carvacrol, and then *p*-cymene, thymol, and γ-terpinene, which occur at low concentration. It should be mentioned that *p*-cymene and γ-terpinene are the precursors of carvacrol and thymol (Kintzios 2003; Burt 2004). These compounds of OEO released from the film matrix result in the elimination of bacteria. Carvacrol and thymol have a phenolic hydroxyl at a different location in the phenolic ring. The hydroxyl group increases their hydrophilic ability, which could help them dissolve in microbial membrane and impair them (Xu *et al.* 2008). In general, the antimicrobial mechanism of

action is considered to disturb the cytoplasmic membrane and disrupt the proton motive force, electron flow, active transport and coagulation of cell contents (Burt 2004).

As previously mentioned, the blend film without OEO showed a good inhibitory activity against TCV, psychrotrophic bacteria, especially *Pseudomonas* spp. population. As gelatin forms 75% of the film, observation of this effect could be attributed to excellent barrier properties against oxygen (Chiou *et al.* 2008). Gómez-Estaca *et al.* (2010) and Ahmad *et al.* (2012) have postulated that the films containing gelatin could have peptides with antimicrobial activity. However, the results obtained in the present experiments were not in agreement with those of Frangos *et al.* (2010), reporting that the blend film did not show any significant ($P > 0.05$) inhibitory activity on the LAB and Enterobacteriaceae.

In addition to the protective effect of OEO film and the antimicrobial activity of OEO in reducing bacterial growth, chemical composition of fish and its species might be effective in reducing the growth of bacteria. For example, the high fat content of fish appears to markedly reduce the action of EOs against various microorganisms in meat products (Mahmoud and Miyashita 2010; Tajkarimi *et al.* 2010).

TVB-N, as an indicator of meat deterioration (Günlü and Koyun 2012), is related to the growth of microorganisms and basic compound formation from their metabolism (Núñez-Flores *et al.* 2013). A TVB-N value of 35 mg N/100 g has been established as an upper acceptable limit for spoilage initiation for fresh fish by the European Commission (CEC, 1995). Initially, TVB-N indicated that the rainbow trout was of good quality, which was in agreement with relatively low initial TVC (2.5 log cfu/g). Lower TVB-N values in the samples wrapped with OEO–blend film could be attributed to the antimicrobial properties of OEO. TVB-N measure grew by increasing the activity of spoilage bacteria and endogenous enzymes (Günlü and Koyun 2012). Similar results have been reported for rainbow trout fillets wrapped with quince seed mucilage films incorporated with oregano, grass carp muscle covered with gelatin-chitosan film enriched with oregano and oregano on anchovy (Günlü and Koyun 2012; Bensid *et al.* 2014; Jouki *et al.* 2014; Wu *et al.* 2014).

pH influences spoilage because of its effect on the microorganism and enzyme activity (Ashie *et al.* 1996). The increase of pH in the OEO–blend film was slower than that in the control samples and the slices treated with blend film due to the reduction of microorganism growth and activity of enzymes. In general, pH increased due to the activity of proteolytic and lipolytic bacteria in the fish (López-Caballero *et al.* 2007).

The results of the present study indicated that the application of OEO–blend film treatment maintained the quality of the rainbow trout slices better than the control and slices wrapped with the neat blend film. Application of the blend film-enriched OEO had the highest antimicrobial effect on psychrotrophic bacteria, TVC and Enterobacteriaceae. The blend film without OEO, due to the restricted access to oxygen, significantly ($P < 0.05$) reduced the growth of *Pseudomonas* spp. counts. Also, lower increasing rate of the TVB-N and pH values in the slices treated with OEO–blend film, compared with the other treatments, confirmed its good antimicrobial properties.

ACKNOWLEDGMENT

The authors would like to thank Salam A. Ibrahim for his valuable guidance and scientific editing.

REFERENCES

AHMAD, M., BENJAKUL, S., SUMPAVAPOL, P. and NIRMAL, N.P. 2012. Quality changes of sea bass slices wrapped with gelatin film incorporated with lemongrass essential oil. Int. J. Food Microbiol. *155*, 171–178.

ALBOOFETILEH, M., REZAEI, M., HOSSEINI, H. and ABDOLLAHI, M. 2013. Effect of montmorillonite clay and biopolymer concentration on the physical and mechanical properties of alginate nanocomposite films. J. Food Eng. *117*, 26–33.

ARASHISAR, Ş., HISAR, O., KAYA, M. and YANIK, T. 2004. Effects of modified atmosphere and vacuum packaging on microbiological and chemical properties of rainbow trout (*Oncorhynchus mykiss*) fillets. Int. J. Food Microbiol. *97*, 209–214.

ASHIE, I., SMITH, J., SIMPSON, B. and HAARD, N.F. 1996. Spoilage and shelf-life extension of fresh fish and shellfish. Crit. Rev. Food Sci. Nutr. *36*, 87–121.

ATREA, I., PAPAVERGOU, A., AMVROSIADIS, I. and SAVVAIDIS, I. 2009. Combined effect of vacuum-packaging and oregano essential oil on the shelf-life of Mediterranean octopus (*Octopus vulgaris*) from the Aegean Sea stored at 4°C. Food Microbiol. *26*, 166–172.

BAE, H.J., PARK, H.J., HONG, S.I., BYUN, Y.J., DARBY, D.O., KIMMEL, R.M. and WHITESIDE, W.S. 2009. Effect of clay content, homogenization RPM, pH, and ultrasonication on mechanical and barrier properties of fish gelatin/montmorillonite nanocomposite films. LWT – Food Sci. Technol. *42*, 1179–1186.

BENAVIDES, S., VILLALOBOS-CARVAJAL, R. and REYES, J. 2012. Physical, mechanical and antibacterial properties of alginate film: Effect of the crosslinking degree and oregano essential oil concentration. J. Food Eng. *110*, 232–239.

BENSID, A., UCAR, Y., BENDEDDOUCHE, B. and ÖZOGUL, F. 2014. Effect of the icing with thyme, oregano and clove extracts on quality parameters of gutted and beheaded anchovy (*Engraulis encrasicholus*) during chilled storage. Food Chem. *145*, 681–686.

BURT, S. 2004. Essential oils: Their antibacterial properties and potential applications in foods – a review. Int. J. Food Microbiol. *94*, 223–253.

CEC. 1995. Commission of the European Community. *Decision 95/149/EC of 8 March 1995 Fixing the Total Volatile BasicNitrogen (TVB-N) Limit Values for Certain Categories of Fishery Products and Specifying the Analysis Methods to be Used*, CEC, Brussels.

CHIOU, B.-S., AVENA-BUSTILLOS, R.J., BECHTEL, P.J., JAFRI, H., NARAYAN, R., IMAM, S.H., GLENN, G.M. and ORTS, W.J. 2008. Cold water fish gelatin films: Effects of cross-linking on thermal, mechanical, barrier, and biodegradation properties. Eur. Polym. J. *44*, 3748–3753.

CHOULIARA, E., KARATAPANIS, A., SAVVAIDIS, I. and KONTOMINAS, M. 2007. Combined effect of oregano essential oil and modified atmosphere packaging on shelf-life extension of fresh chicken breast meat, stored at 4C. Food Microbiol. *24*, 607–617.

DALGAARD, P. 2003. FISH | Spoilage of seafood. In *Encyclopedia of Food Sciences and Nutrition*, 2nd Ed. (Editor-in-Chief C. Benjamin, ed.) pp. 2462–2471, Academic Press, Oxford, UK.

EMIROĞLU, Z.K., YEMIŞ, G.P., COŞKUN, B.K. and CANDOĞAN, K. 2010. Antimicrobial activity of soy edible films incorporated with thyme and oregano essential oils on fresh ground beef patties. Meat Sci. *86*, 283–288.

FRANGOS, L., PYRGOTOU, N., GIATRAKOU, V., NTZIMANI, A. and SAVVAIDIS, I.N. 2010. Combined effects of salting, oregano oil and vacuum-packaging on the shelf-life of refrigerated trout fillets. Food Microbiol. *27*, 115–121.

GÓMEZ-ESTACA, J., LÓPEZ DE LACEY, A., LÓPEZ-CABALLERO, M.E., GÓMEZ-GUILLÉN, M.C. and MONTERO, P. 2010. Biodegradable gelatin–chitosan films incorporated with essential oils as antimicrobial agents for fish preservation. Food Microbiol. *27*, 889–896.

GOULAS, A.E. and KONTOMINAS, M.G. 2005. Effect of salting and smoking-method on the keeping quality of chub mackerel (*Scomber japonicus*): biochemical and sensory attributes. Food Chem. *93*, 511–520.

GRAM, L. and DALGAARD, P. 2002. Fish spoilage bacteria – problems and solutions. Curr. Opin. Biotechnol. *13*, 262–266.

GRAM, L. and HUSS, H.H. 1996. Microbiological spoilage of fish and fish products. Int. J. Food Microbiol. *33*, 121–137.

GÜNLÜ, A. and KOYUN, E. 2012. Effects of vacuum packaging and wrapping with chitosan-based edible film on the extension of the shelf life of sea bass (*Dicentrarchus labrax*) fillets in cold storage (4°C). Food Bioprocess Technol. *6*, 1713–1719.

GYAWALI, R. and IBRAHIM, S.A. 2014. Natural products as antimicrobial agents. Food Control *46*, 412–429.

IBRAHIM SALLAM, K. 2007. Antimicrobial and antioxidant effects of sodium acetate, sodium lactate, and sodium citrate in refrigerated sliced salmon. Food Control *18*, 566–575.

ICMSF. 2002. *Microorganisms in Foods 7*, Kluwer Academic/Plenum Publishers, New York, NY.

ITURRIAGA, L., OLABARRIETA, I. and DE MARAÑÓN, I.M. 2012. Antimicrobial assays of natural extracts and their inhibitory effect against *Listeria innocua* and fish spoilage bacteria, after incorporation into biopolymer edible films. Int. J. Food Microbiol. *158*, 58–64.

JEON, Y.-J., KAMIL, J.Y. and SHAHIDI, F. 2002. Chitosan as an edible invisible film for quality preservation of herring and Atlantic cod. J. Agric. Food Chem. *50*, 5167–5178.

JOUKI, M., YAZDI, F.T., MORTAZAVI, S.A., KOOCHEKI, A. and KHAZAEI, N. 2014. Effect of quince seed mucilage edible films incorporated with oregano or thyme essential oil on shelf life extension of refrigerated rainbow trout fillets. Int. J. Food Microbiol. *174*, 88–97.

KARABAGIAS, I., BADEKA, A. and KONTOMINAS, M.G. 2011. Shelf life extension of lamb meat using thyme or oregano essential oils and modified atmosphere packaging. Meat Sci. *88*, 109–116.

KINTZIOS, S.E. 2003. *Oregano: The Genera Origanum and Lippia*, CRC Press, Boca Raton, FL, USA.

KOSTAKI, M., GIATRAKOU, V., SAVVAIDIS, I.N. and KONTOMINAS, M.G. 2009. Combined effect of MAP and thyme essential oil on the microbiological, chemical and sensory attributes of organically aquacultured sea bass (*Dicentrarchus labrax*) fillets. Food Microbiol. *26*, 475–482.

LÓPEZ DE LACEY, A., LÓPEZ-CABALLERO, M. and MONTERO, P. 2013. Agar films containing green tea extract and probiotic bacteria for extending fish shelf-life. LWT – Food Sci. Technol. *55*, 559–564.

LÓPEZ-CABALLERO, M.E., MARTÍNEZ-ALVAREZ, O., GÓMEZ-GUILLÉN, M.D.C. and MONTERO, P. 2007. Quality of thawed deepwater pink shrimp (*Parapenaeus longirostris*) treated with melanosis-inhibiting formulations during chilled storage. Int. J. Food Sci. Technol. *42*, 1029–1038.

LYHS, U., LAHTINEN, J., FREDRIKSSON-AHOMAA, M., HYYTIÄ-TREES, E., ELFING, K. and KORKEALA, H. 2001. Microbiological quality and shelf-life of vacuum-packaged "gravad" rainbow trout stored at 3 and 8C. Int. J. Food Microbiol. *70*, 221–230.

MAHMOUD, B.S. and MIYASHITA, K. 2010. *Essential Oils: Natural Antimicrobials for Fish Preservation. Handbook of Seafood Quality, Safety and Health Applications*, pp. 217–225, John Wiley & Sons, New York, USA.

MAIZURA, M., FAZILAH, A., NORZIAH, M.H. and KARIM, A.A. 2007. Antibacterial activity and mechanical properties of partially hydrolyzed sago starch–alginate edible film containing lemongrass oil. J. Food Sci. *72*, 324–330.

MEXIS, S., CHOULIARA, E. and KONTOMINAS, M. 2009. Combined effect of an oxygen absorber and oregano essential oil on shelf life extension of rainbow trout fillets stored at 4C. Food Microbiol. *26*, 598–605.

NOWZARI, F., SHÁBANPOUR, B. and OJAGH, S.M. 2013. Comparison of chitosan-gelatin composite and bilayer coating and film effect on the quality of refrigerated rainbow trout. Food Chem. *141*, 1667–1672.

NÚÑEZ-FLORES, R., GIMÉNEZ, B., FERNÁNDEZ-MARTÍN, F., LÓPEZ-CABALLERO, M., MONTERO, M. and GÓMEZ-GUILLÉN, M. 2013. Physical and functional characterization of active fish gelatin films incorporated with lignin. Food Hydrocolloids *30*, 163–172.

OJAGH, S.M., REZAEI, M., RAZAVI, S.H. and HOSSEINI, S.M.H. 2010. Development and evaluation of a novel biodegradable film made from chitosan and cinnamon essential oil with low affinity toward water. Food Chem. *122*, 161–166.

PYRGOTOU, N., GIATRAKOU, V., NTZIMANI, A. and SAVVAIDIS, I.N. 2010. Quality assessment of salted, modified atmosphere packaged rainbow trout under treatment with oregano essential oil. J. Food Sci. *75*, M406–M411.

RHIM, J.-W. 2004. Physical and mechanical properties of water resistant sodium alginate films. LWT – Food Sci. Technol. *37*, 323–330.

ROYO, M., FERNÁNDEZ-PAN, I. and MATÉ, J.I. 2010. Antimicrobial effectiveness of oregano and sage essential oils incorporated into whey protein films or cellulose-based filter paper. J. Sci. Food Agric. *90*, 1513–1519.

SALGADO, P.R., LÓPEZ-CABALLERO, M.E., GÓMEZ-GUILLÉN, M.C., MAURI, A.N. and MONTERO, M.P. 2013. Sunflower protein films incorporated with clove essential oil have potential application for the preservation of fish patties. Food Hydrocolloids *33*, 74–84.

TAJKARIMI, M., IBRAHIM, S. and CLIVER, D. 2010. Antimicrobial herb and spice compounds in food. Food Control *21*, 1199–1218.

WU, J., GE, S., LIU, H., WANG, S., CHEN, S., WANG, J., LI, J. and ZHANG, Q. 2014. Properties and antimicrobial activity of silver carp (*Hypophthalmichthys molitrix*) skin gelatin -chitosan films incorporated with oregano essential oil for fish preservation. Food Packag. Shelf Life *2*, 7–16.

XU, J., ZHOU, F., JI, B.P., PEI, R.S. and XU, N. 2008. The antibacterial mechanism of carvacrol and thymol against *Escherichia coli*. Lett. Appl. Microbiol. *47*, 174–179.

ZINOVIADOU, K.G., KOUTSOUMANIS, K.P. and BILIADERIS, C.G. 2009. Physico-chemical properties of whey protein isolate films containing oregano oil and their antimicrobial action against spoilage flora of fresh beef. Meat Sci. *82*, 338–345.

ZIVANOVIC, S., CHI, S. and DRAUGHON, A.F. 2005. Antimicrobial activity of chitosan films enriched with essential oils. J. Food Sci. *70*, M45–M51.