

The Effect of Sodium Alginate Coating Incorporated with Lactoperoxidase System and *Zataria multiflora boiss* Essential Oil on Shelf Life Extension of Rainbow Trout Fillets During Refrigeration

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ABSTRACT: The present study is aimed at evaluating the effect of functional alginate coating incorporated with Lactoperoxidase (LPOS) and *Zataria multiflora* Essential Oil (ZEO) or both as natural additives on microbial and chemical characteristics of rainbow trout fillets. Firstly, Different treatments of trout fillets were prepared using ZEO (0.5 and 1%) and LPOS (5%) individually and in combination. Then stored in the refrigerator for 16 days and were analyzed for chemical (pH and Total Volatiles Base-Nitrogen (TVB-N)) and microbial (Total mesophilic viable count, psychotropic count, *Shewanella putrefaciens* count, and *Pseudomonas* spp. count) characteristics at 4-day intervals. Results indicated that the combination of ZEO and LPOS had the strongest effect on chemical spoilage parameters (TVN, pH) and spoilage microbial flora of trout fillets during storage; however, samples with the individual use of ZEO or LPOS also had statistically significant ($P < 0.05$) effects on preserving the chemical and microbial quality of trout fillets. According to the results, the application of sodium alginate coating impregnated with LPOS and ZEO especially in combination can control undesirable chemical and microbial changes of fish and extend its shelf life during refrigeration.

KEYWORDS: Rainbow trout; Sodium alginate coating; Lactoperoxidase system; *Zataria multiflora* essential oil; Shelf life.

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INTRODUCTION

Fish is a highly perishable food and become spoiled faster than meat. Fish spoilage can be classified into two general categories; bacterial and chemical (autolytic). The spoilage decreases the quality of proteins in fish [1]. Cold storage and freezing are used to control or to reduce these changes in fish. However, these techniques cannot completely prevent microbial or chemical spoilage [2,3]. Rainbow trout is one of the best-farmed fish with increasing demand in the market. One of the most important features of this fish is its freshness and non-frozen supply. Due to the increasing consumer awareness, there is a bigger demand for fresh fish rather than frozen fish [2-4]; however, given the high perishability of fish, non-frozen storage causes a statistically significant ($P < 0.05$) reduction in shelf life [4]. On the other hand, during the short-term storage of fish, freezing not only imposes high costs but also reduces its marketability [5]. In recent years, numerous studies have been conducted on the use of natural ingredients to maintain quality and to improve fish shelf life [6-12]. Biodegradable substances such as polysaccharides and proteins can be applied to cover the fish fillets to prevent quality changes during frozen storage. So far, film formation and coating solution and their characteristics have mainly focused on polysaccharides such as starch and its derivatives, various microbial and plant resins, chitosan, pectin and alginate [13-15]. Derived from brown algae, Alginate, like starch and cellulose, is a polysaccharide with 1,000 to 3,000 structural units linked to one another, constituting two relatively hard and flexible sections [14]. Gel forming ability, increased texture firmness, stabilizing and film forming ability are some of the properties of alginate [13]. The high film-forming ability of alginate has turned it into an appropriate food coating; however, the presence of antibacterial and antioxidant combinations causes an increase in storage properties [1, 17].

According to the disadvantages of chemical preservatives such as carcinogenic as well as increased public awareness, currently, the negative impression has been developed among the consumers about synthetic food additives [18]. A public tendency has been raised to the use of natural preservatives for shelf life enhancement of food [10]; therefore, nowadays, the use of plant extracts and essential oils (EOs) is regarded as preservatives [12]. Phenolic and flavonoids groups

are the main reason for antioxidant and antimicrobial activities of plant extracts and Eos [17]. Electron microscopic images showed that these lipophilic compounds act on the cell membrane, and cause substantial morphological damages and changes its permeability leading to the release of cell contents [19]. *Zataria multiflora* EO (ZEO) is a known plant essential oil with antioxidant, antimicrobial and antifungal properties, and its major compounds include terpenes such as carvacrol, thymol and p-cymene [20].

Lactoperoxidase Enzyme (LPO) is a single-chain polypeptide found in milk, saliva, and tears which are secreted by glands in mammals. This enzyme prolongs the storage duration of raw milk and its products [21]. It has been introduced as one of the most important enzymes widely used in food industries as an antimicrobial agent, particularly in food packaging industry [22]. This enzyme has a bactericidal effect on gram-positive and gram-negative bacteria as well as antifungal and antiviral activities [4]. LPO catalyzes the oxidation of thiocyanate via hydrogen peroxide and produces intermediate oxidizing compounds with short lifespans such as hypothiocyanate (OSCN-) and oxyacids of thiocyanate with antimicrobial effects [23]. LPOS consists of LPO, potassium thiocyanate and hydrogen peroxide [23].

Heretofore, there are few studies which have used alginate coating solutions in fish [24-26], and no study was conducted on the effects of the combinational use of LPOS and ZEO on shelf life extension of food. Therefore the present study was performed to evaluate the ability of sodium alginate coating containing LPOS and ZEO to preserve the microbial and chemical quality of fresh trout fillets during refrigeration

EXPERIMENTAL SECTION

Materials

LPOS was composed of lactoperoxidase (LPO; 120 U/mg Sigma–Aldrich, Steinheim, Germany), glucose oxidase (GO; Sigma–Aldrich); D-(α)-glucose (Glu; Sigma–Aldrich), and potassium thiocyanate (KSCN; Bioserae, Mont oliou, France)(4). Alginate (medium molecular weight, 85% degree of deacetylation) was obtained from Sigma–Aldrich. Glycerol was purchased from Fisher Scientific Inc. (Merck, Darmstadt, Germany). Cultures media including Nutrient Agar (NA) and Plate Count Agar (PCA) were purchased from Merck (Darmstadt, Germany) and Iron agar was purchased from

LYNGBY (Laboratorios Conda, Madrid, Spain). ZEO (was purchased from Iranian Institute of Medicinal Plants, Karaj, Alborz province, Iran) and tween 80 (sigma-aldrich). Magnesium oxide, boric acid, methyl red, methylene blue, and ethanol were obtained from Merck, Darmstadt, Germany.

Preparation of rainbow trout fillets

Fish trout fillets were prepared from fresh *Oncorhynchus mykiss* (average weight: 300±50 g) which were obtained from a local farm located in Mashhad, Iran (August- September 2015), and they were immediately in ice cold water transported to the laboratory within 12 h of harvesting (Food Hygiene Department, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad), and then were washed to remove the remaining blood and slimes. The fish were eviscerated, headed and filleted (approximately every fillet of uniform 21× 9 cm² and weight 100g) by hand. Skin and spiny bones were removed as possible as by hand from fillet. Fillet samples were randomly divided into seven treatment according to Table 1.

Preparation of LPOS

LPOS was prepared according to the method previously described (4). LPOS components (weight ratios: 1.00, 0.35, 108.70 and 1.09 for the LPO, glucose oxidase, D-(α)-glucose, potassium thiocyanate, and H₂O₂, respectively) were dissolved in phosphate buffer (50mL, pH 6.2) based on 15.5 mg of LPO. The solution filter-sterilized (0.2 micron) and incubated at 23 °C for 24 h under constant shaking (160 rpm) by a shaker incubator (GFL 3031, Burgwedel, Germany) to boost the antimicrobial activity of LPOS.

Preparation of coating solutions and treatments

Alginate solutions were prepared by dissolving the alginate powder (3% w/v) in sterilized distilled water containing 2% glycerol as a plasticizer to improve coating flexibility at a controlled temperature (70 °C) and were constantly stirred for 30 min until it becomes clear. Calcium chloride was dissolved (2% w/v) in distilled water and sterilized by autoclaving at 121 °C for 15 min. Then the boosted LPOS (5%) and ZEO (0.5 and 1%) were added to the solutions. ZEO was dissolved in the alginate, solutions using tween 80 (0.2 g/g EO)

at a controlled temperature (40 °C) and then stirred for 30 min to create a uniform, stable and clear solution. Trout fillets were divided into seven groups as treatments which are described in Table 1. Then immersed in alginate solutions (1 min), drained (30 s), immersed in a CaCl₂ solution (30 s), and were stored at 4 ± 1 °C for 16 days to be analyzed at 4-day intervals: 0, 4, 8, 12 and 16 (4, 10).

Microbiological analysis

Fish fillet samples without skin (25g) were diluted with 225 ml sterile peptone water (0.1%) and homogenized by a stomacher (Seward Ltd, London Co, UK). Serial dilutions of the homogenates were then prepared, and the amounts of 10 μL (4, 26) were plated on specific agars including; NA for enumeration of total mesophilic viable counts (TMVC) at 30 °C for 48h (27), PCA for enumeration of psychrotrophic bacteria at 7 °C for 10 days (6), and Iron agar for enumeration of *Shewanella putrefaciens* (black colonies) and *Pseudomonas fluorescens* (white colonies) at 30 °C for 3–4 days (4).

Total volatile basic nitrogen

TVB-N is one of the most widely used indices of seafood quality. It is a general term which includes the measurement of trimethylamine (produced by spoilage bacteria), dimethylamine (produced by autolytic enzymes during storage), ammonia (produced by the deamination of amino-acids and nucleotide catabolites) and other volatile basic nitrogenous compounds associated with sea food spoilage [29]. Micro-diffusion method was used to measure TVB-N value of the samples (22). Briefly, homogenized samples were mixed with magnesium oxide (MgO) and then distilled. The distillates were collected in flasks containing an aqueous solution of boric acid (3% v/v) and an indicator solution produced by dissolving methyl red (0.1 g) and methylene blue (0.1 g) in ethanol (100 mL of). The boric acid solution was titrated with a sulfuric acid solution (0.05 mol/L). The TVB-N value was calculated according to the consumption of sulfuric acid and was expressed as mg 100 g⁻¹ fish flesh.

Determination of pH

Fish samples (10 g) were homogenized in 100 mL of distilled water at room temperature. Mixtures were then

Table 1: List of combinations and treatments.

Treatment	Description	
1	CON	Samples without any coating solution
2	ALG	Samples coated with alginate solution
3	E1	Samples coated with alginate solution containing 0.5 % ZEO
4	E2	Samples coated with alginate solution containing 1 % ZEO
5	L	Samples coated with alginate solution containing LPOS
6	LE1	Samples coated with alginate solution containing LPOS and 0.5 % ZEO
7	LE2	Samples coated with alginate solution containing LPOS and 1 % ZEO

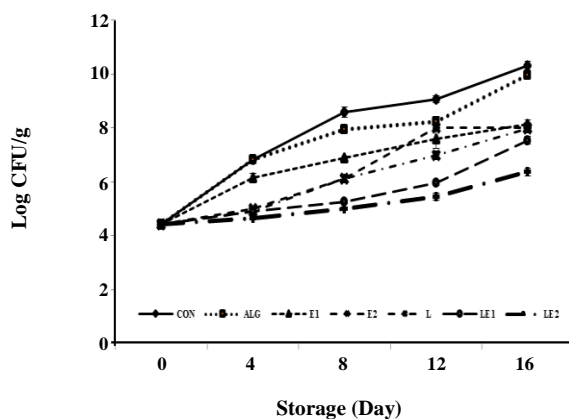


Fig. 1: Changes in TMVC of trout fillets as affected by alginate coating containing ZEO (0.5 and 1%) and LPOS (5%) during refrigeration (4 ± 1 °C). Values are the mean \pm SD (CON: without coating, ALG: alginate coating, E1: alginate coating + 0.5 % ZEO, E2: alginate coating + 1 % ZEO, L: alginate containing + LPOS, LE1: alginate containing + LPOS and 0.5 % ZEO, LE2: alginate containing + LPOS and 1 % ZEO)

filtered, and pH values of filtrates were measured using a digital pH meter (Cyberscan PC 510 UK) (4).

Statistical analysis

All the tests of the present study were performed in five replicates. Mean standard deviation, minimum and maximum log of the bacterial count and chemical values were reported for different groups on days 0, 4, 8, 12, 16. Comparison of the mean log of the bacterial count and chemical values among the groups during the 16-day period were analyzed by repeated measure ANOVA followed by Dunnett-T3 post-hoc tests. All statistical analysis performed by SPSS version 21 and $P < 0.05$ were considered as statistically significant ($P < 0.05$).

RESULTS AND DISCUSSION

Changes in the microbial flora of fish fillet Total Mesophilic Viable Count (TMVC)

Changes of TMVC during the storage period are shown in Fig. 1. According to the International Commission on Microbiological Specifications for Foods, most fresh aquatic animals have a range of 2-5 Log CFU/g for TMVC (27). The results of the investigation on TMVC showed that ZEO, either alone or in combination with LPOS, is potent in controlling the growth of microorganisms.

As can be seen in Fig. 1, the initial value of TMVC was 4.40 Log CFU/g for all the treatments, which indicates a high quality of the used fillets, and it is in line with results of former studies (1, 2, 4). Maximum allowance level of TMVC is 7.00 Log CFU/g for rainbow trout (40). Within the first 8 days of storage, TMVC of treated samples was less than 8.00 Log CFU/g, while it reached 8.57 Log CFU/g in control. TMVC has increased in all treatments during the storage which was consistent with other studies (2, 4, 5). Control samples showed the highest increase rate in TMVC during the storage and reached 10.29 Log CFU/g at the end of the storage; while samples containing ZEO incorporated with LPOS (LE2) had the lowest increase rate in TMVC.

TMVC values of all the treated samples were statistically significant ($P < 0.05$) lower than CON and ALG samples. Hamzeh and Rezaei (2011) investigated the effects of sodium alginate on the quality of rainbow trout fillets stored at 4 ± 2 °C and showed that treated samples had the lowest increase rate in TMVC (2). Another study by Shokri et al. (2014) investigated the efficacy of LPOS-whey protein coating on the shelf-life extension of rainbow trout fillets during cold storage, and they have noted that treating with LPOS could increase shelf-life

of rainbow trout fillets by reducing TMVC increase rate [4]. Shakeri *et al.* showed that ZEO, while incorporated with whey protein-based films, could decrease TMVC in swordfish fillets [26]. Similar results were obtained from other studies as well [5, 28, 29]. Other researchers also showed that combinational use of natural preservatives could decrease TMVC value [29, 30]. Elotmani *et al.* (2004) used nisin and LPOS in combination for inhibition of the microbial flora of fish. Their result indicated that the combined use of nisin (100 IU/mL) and LPOS (level 10) was statistically significant ($P < 0.05$) more effective than their individual use [31]. Van Haute *et al.* (2016) also illustrated that combination use of cinnamon, oregano and thyme essential oils had a higher effect on the microbial quality of fish [32]. In the present study, combinational use of LPOS and ZEO had a higher effect compared to other samples on reducing TMVC increase rate.

On the other hand, individual use of LPOS is more effective in reducing the increase rate of TMVC than individual use of ZEO showing the higher antimicrobial activity of LPOS than ZEO. It can be due to the presence of glucose oxidase in LPOS that continuously abets oxidizing products, leading to extended bactericidal effects.

Psychrotrophic count:

Psychrotrophic bacteria are known as different species of bacteria able to grow at a temperature of 7°C or even less. They are the major microorganisms responsible for spoilage in the aerobic storage of various types of meat products. The permitted bacterial load has been reported 7 Log CFU/g for psychrotrophic aerobic bacteria [33]. The effects of treatments on psychrotrophic bacterial count have been shown in Fig. 2. The initial count of Psychrotrophic bacteria in the samples was about 5 log CFU/g, which was in the same range as previous studies [4, 22, 34]. The psychrotrophic count increased during the storage and exceeded the permitted limit (10^7 CFU g⁻¹) in CON, ALG and E1 samples at Day 8; in E2 samples at Day 12, and in L and LE1 at Day 16. Although LE2 samples did not reach the permitted limit during the storage. Psychrophilic count of the treated samples was statistically significant ($P < 0.05$) lower than CON and ALG at the end of the storage. The lowest psychrotrophic count was observed in LE2 samples, while the highest psychrotrophic count was observed

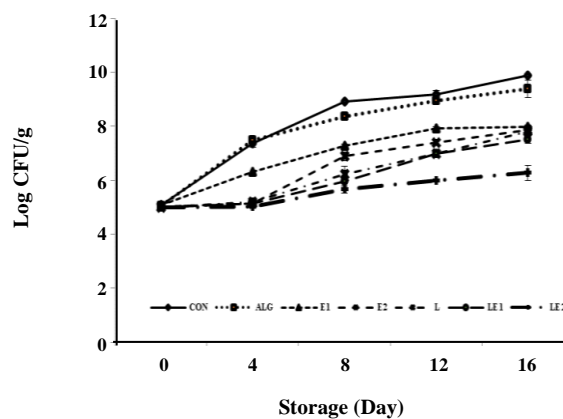


Fig. 2: Changes in the Psychrotrophic count of trout fillets as affected by alginate coating containing ZEO (0.5 and 1%) and LPOS (5%) during refrigeration (4 ± 1 °C). Values are the mean \pm SD (CON: without coating, ALG: alginate coating, E1: alginate coating + 0.5 % ZEO, E2: alginate coating + 1 % ZEO, L: alginate containing + LPOS, LE1: alginate containing + LPOS and 0.5 % ZEO, LE2: alginate containing + LPOS and 1 % ZEO)

in CON and ALG samples. These results are consistent with findings of previous studies (5, 19). A higher psychrophilic count was observed in samples containing a lower level of ZEO (E1 and LE1) compared to samples containing a higher level of ZEO (E2 and LE2). Zolfaghari *et al.* (2011) reported that extracts of ZEO are highly potent in controlling the growth of psychrotrophic microorganisms [18]. Hamzeh and Rezaei (2011) indicated that psychrotrophic bacterial load had increased during the storage in all treatments, with a higher gradient in control Samples (2). Similar results were observed in the present study as well. In the present study, the combinational use of LPOS and ZEO had a higher effect on reducing the increasing rate of the psychrotrophic count. In the current study, samples containing LPOS individually (L) showed fewer psychrotrophic count in comparison with control as well as treatments containing ZEO individually (E1 and E2). Therefore these findings are in line with the study of Shokri *et al.* (2014) who reported lower psychrotrophic bacterial count in treatments containing 5% and 7.5% concentrations of LPOS(4). Jasoor *et al.* (2014) also reported similar results regarding the effect of LPOS treated samples on the reduction of the psychrotrophic count in rainbow trout fillets [20].

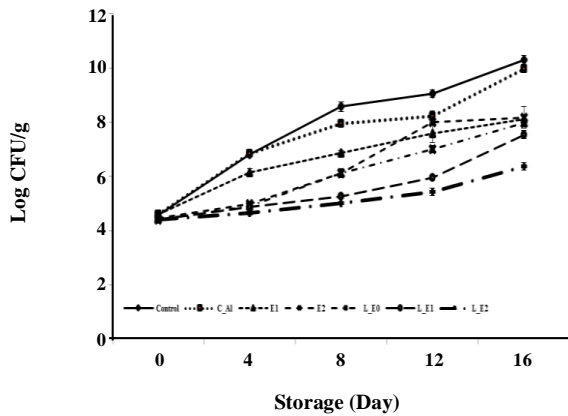


Fig. 3: Changes in *Pseudomonas* spp. of trout fillets as affected by alginate coating containing ZEO (0.5 and 1%) and LPOS (5%) during refrigeration (4 ± 1 °C). Values are the mean \pm SD (CON: without coating, ALG: alginate coating, E1: alginate coating + 0.5 % ZEO, E2: alginate coating + 1 % ZEO, L: alginate containing + LPOS, LE1: alginate containing + LPOS and 0.5 % ZEO, LE2: alginate containing + LPOS and 1 % ZEO).

Pseudomonas spp

Pseudomonas spp. are gram-negative bacteria that are the main reason of spoilage in cold storage conditions such as proteolysis, as the leading cause of spoilage which starts when glucose and gluconate contents are depleted, and bacterial count reaches 7 to 8 Log CFU/g (20). As shown in Fig. 3, an initial count of *Pseudomonas* spp. was 4.00- 4.60 Log CFU/g, which has increased during the storage time; and has reached to a final population of 10.29, 9.97, 8.12, 8.17, 7.97, 7.53 and 6.36 Log CFU/g, for the groups of CON, ALG, E1, E2, L, LE1, and LE2, respectively. All treated samples had statistically significant ($P < 0.05$) lower *Pseudomonas* spp. count when compared to CON and ALG samples. Combinational use of LPOs and ZEO had a higher effect on the reduction of the increase rate of *Pseudomonas* spp. count than their individual use ($P < 0.05$); and individual use of LOPS was more effective than ZEO (0.5% and 1%). These results are in line with findings of other researchers (4, 5, 10, 19, 28).

Shewanella putrefaciens count

S. putrefaciens is a gram-negative spoilage bacteria in fish, particularly under cold storage condition (4). As indicated in Fig. 4, *S. putrefaciens* count of all the samples increased during the storage time ($p < 0.001$). All treated samples had

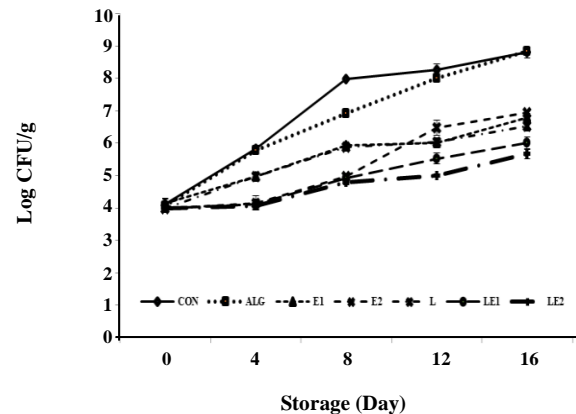


Fig. 4: Changes in *S. putrefaciens* count of trout fillets as affected by alginate coating containing ZEO (0.5 and 1%) and LPOS (5%) during refrigeration (4 ± 1 °C). Values are the mean \pm SD (CON: without coating, ALG: alginate coating, E1: alginate coating + 0.5 % ZEO, E2: alginate coating + 1 % ZEO, L: alginate containing + LPOS, LE1: alginate containing + LPOS and 0.5 % ZEO, LE2: alginate containing + LPOS and 1 % ZEO).

statistically significant ($P < 0.05$) lower *S. putrefaciens* count than CON and ALG samples. Among the treated samples, the lowest *S. putrefaciens* counts were observed in LE2, LE1, L, E2, and E1, respectively. The final counts of *S. putrefaciens* were 8.80, 8.84, 6.94, 6.79, 6.51, 6.01 and 5.66 Log CFU/g, for CON, ALG, E1, E2, LE, LE1, and LE2. Results also indicated that individual use of LOPS was more effective than individual use of different concentration of ZEO. Concurrent use of LPOS and ZEO also resulted in less bacterial counts than their individual use during the storage time. To the best of our knowledge, there has been no study regarding the effect of the combinational use of LPOS and ZEO. However, Shokri et al. (2014) evaluated the effect of whey protein coating incorporated with LPOS in trout fillets and reported that treated samples had lower *S. putrefaciens* count when compared to control. Jasoore et al. (2014) reported the same results about the effect of LPOS on *S. putrefaciens* count as well. There is no previous study on the changes of *Shewanella* spp. count in samples treated by ZEO but Kostaki et al. (2009) indicated a notable sensitivity of *S. putrefaciens* to thyme EO (35), which is entirely consistent with the results of the present study.

Changes in chemical characteristics

TVN changes

TVN is a product derived from bacterial spoilage, and endogenous enzymes activities and its content is

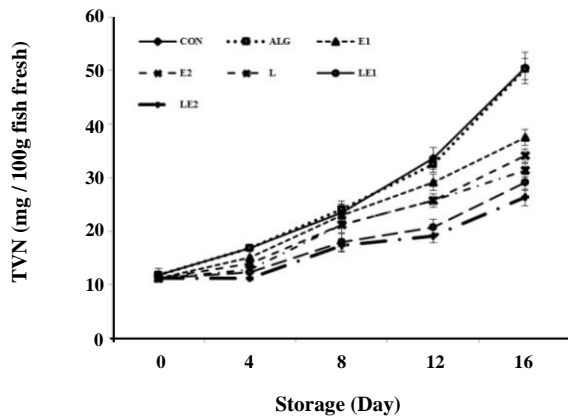


Fig. 5: Changes in TVN of trout fillets as affected by alginate coating containing ZEO (0.5 and 1%) and LPOS (5%) during refrigeration (4 ± 1 °C). Values are the mean \pm SD (CON: without coating, ALG: alginate coating, E1: alginate coating + 0.5 % ZEO, E2: alginate coating + 1 % ZEO, L: alginate containing + LPOS, LE1: alginate containing + LPOS and 0.5 % ZEO, LE2: alginate containing + LPOS and 1 % ZEO).

considered as an indicator of spoilage and destruction of fish (4). As it can be realized from Fig. 5, the range of 11.2 to 11.7 mg/100 g of fish muscle was observed in raw fillets at baseline (day 0). Results of the present study were similar to other studies indicating TVN values in a range of 5-20 mg/100g fresh fish (2). TVN values increased during the storage in all samples ($p < 0.05$), although the incorporation of ZEO and LPOS or both with sodium alginate coating has led to a statistically significant ($P < 0.05$) protection against TVN formation compared to CON and ALG groups, which were caused by antimicrobial effects of ZEO and LPOS or both. Values of 30-40 mg TVN/100g, as the maximum acceptable limit for fish muscle, have been previously and continuously reported by many researchers [4, 5, 36, 37]. In the present study, the mean values of TVN in E1, E2, L, LE1, and LE2 samples were 37.52, 34.16, 31.36, 29.12 and 26.32, respectively on the last day of the storage, which was in the acceptable range. Combinational use of LPOS and ZEO had a higher effect than other samples on the reduction of the increase rate of TVN. Former studies which have used ZEO or thyme EO along with alginate [38], whey protein [33], carboxymethyl cellulose [5] as a coating solution, reported similar results of TVN values with the present study. Hamzeh and Rezaie (2011) investigated antioxidant and antibacterial effects of sodium alginate enriched with thyme EO on rainbow trout fillets during

cold storage and reported that TVN values of the samples treated with sodium alginate enriched with 1% and 1.5% thyme EO were less than other treated samples [38]. Raeisi et al. (2014) investigated the effect of carboxymethyl cellulose coating incorporated with ZEO on TVN values of fish during cold storage and reported a statistically significant ($P < 0.05$) decrease in TVN production in fish fillet [5]. Zolfaghari et al. (2010) have used the direct addition of ZEO to extend the shelf life of rainbow trout fillets during cold storage and reported that the use of ZEO was more effective on the reduction of the increase rate of TVN (39). All of these studies were completely consistent with the results obtained in this study. There are few studies on the use of LPOS application along with coating solutions (4, 22). Shokri et al. (2014) used different levels of LPOS with whey protein coating and reported a dose-dependent reduction of TVN formation in fish fillets (4). Jasour et al. (2014) also investigated the effect of chitosan coating containing LPOS and reported that LPOS group had statistically significant ($P < 0.05$) lower TVN values [22]. The results of the above studies were in accordance with the results of the present study as well.

pH changes

Fig. 6 shows pH changes in fish fillets within 16 days of cold storage. Initial pH of all samples was 6.11. Similar to previous studies, pH value of fish fillets slightly decreased at first and then increased [5, 36]. The increase of pH value during the storage of fish fillets indicates accumulation of alkaline compositions such as ammonium and trimethylamine compounds derived from the metabolism of the microorganism [37]. Moreover, pH values of CON and ALG samples increased by 7.12, 6.8, respectively at the end of the storage period which had the highest pH values among all samples. Among treated samples, samples with ZEO 0.5% (E1) and samples containing ZEO 1% + LPOS (LE2) showed the highest and the lowest effect on control of pH value of fresh fish during the storage respectively ($p < 0.05$). In the present study combination use of LPOS and ZEO had a higher effect than their individual use on control of the increase rate of pH. Also, there was no statistically significant ($P < 0.05$) difference in pH values of L, LE1 and LE2 samples during storage. pH changes trend in the present study is similar to other studies conducted to evaluate the effect of ZEO or LPOS in fish (4, 28, 37).

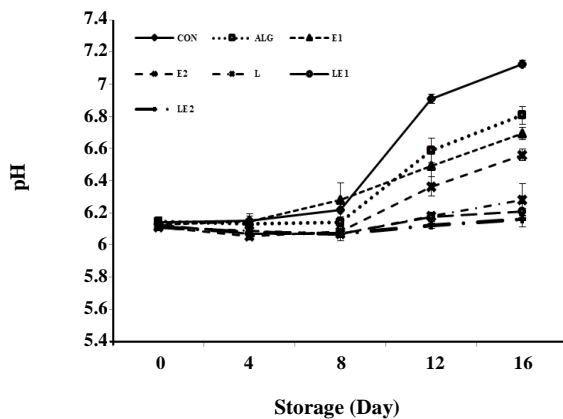


Fig. 6: Changes in pH of trout fillets as affected by alginate coating containing ZEO (0.5 and 1%) and LPOS (5%) during refrigeration (4 ± 1 °C). Values are the mean \pm SD (CON: without coating, ALG: alginate coating, E1: alginate coating + 0.5 % ZEO, E2: alginate coating + 1 % ZEO, L: alginate containing + LPOS, LE1: alginate containing + LPOS and 0.5 % ZEO, LE2: alginate containing + LPOS and 1 % ZEO).

CONCLUSIONS

This study indicates the potential power of LPOS and ZEO alone and in combination in extending the shelf life of fish. The presence of LPOS and ZEO or both could statistically significant ($P < 0.05$) reduce the growth of microbial flora and undesirable chemical changes and could also preserve the quality of fish samples when compared to control. Results also revealed that individual use of LPOS was more effective than individual use of different concentration of ZEO (40). Combinational use of LPOS and ZEO (LE1 and LE2 samples) had a higher effect than their individual use. Finally, ZEO (1%) + LPOS (5%) were more efficient in preserving the quality of fish fillets based on microbial flora counts and chemical (TVN and pH) changes of the samples and could extend the shelf life of fish fillets to 8 days when compared to the control. Therefore, according to the results of the present study and consumer preference for natural additives, sodium alginate coating impregnated with LPOS and ZEO is a proper candidate to be applied in the food industry to control microbial and chemical changes and to extend the shelf life of fish during refrigeration. The sensory evaluation has not been done on fish trout fillets in this study. However in previous and simultaneous studies, food models with similar level from ZEO (38, 40 and 41). and LPOS (4, 5 and 22), have shown that their addition The dietary model has no negative effect on the sensory properties of the product.

Conflict of interests

The authors declare no conflict of interests.

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