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The effect of multilayered chitosan–pectin–*Mentha piperita* and lemon essential oil on oxidation effects and quality of rainbow trout fillet (*Oncorhynchus mykiss*) during refrigeration at 4±1˚C storage

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Abstract

This study was carried out to evaluate the lipid oxidation and quality of rainbow fish fillet coated by multilayer aqueous solution of chitosan–pectin and *Mentha piperita* extract or lemon essential oil in different percentages (0.5 and 1%) at 4±1˚C. The essential oil was extracted by hydrodistillation method. Protein, fat, moisture and ash contents of rainbow trout fillet samples were determined. The control and the coated fish samples were analyzed during 16-day periodically for lipid oxidation expressed by peroxide value (PV) and thiobarbituric acid, meat deterioration by total volatile base nitrogen, free fatty acids and sensory characteristics. The results have shown that *Mentha piperita* 1% was the most appropriate coverage (p<0.05) with 0.5% extracted essential oil. In all samples, *M. piperita* essential oil results for PV, 2-thiobarbituric and free fatty acid (5.15±0.05, 0.23±0.08 5.15±0.05, 1.9±0.03) were better than those of lemon (7.43±0.12, 0.25±0.08 and 2.1±0.05). The sensorial data results have shown that the best time of storage control and covered samples was approximately less than 8 and 16-day, respectively.

Keywords: *Oncorhynchus mykiss*, Lemon, peppermint *Mentha piperita* Oil, Chitosan, Oxidation.

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Introduction

Fish is an extremely delicate food compared with other fresh commodities. Therefore, its marketing traditionally focused on frozen and processed products (Bahmani *et al*., 2014). Freezing is a common preservation method used to control or decrease biochemical changes in fish but it does not completely inhibit chemical reactions (for example, lipid oxidation) that lead to quality deterioration of fish (Sathivel, 2005). Fish is more prone to lipid oxidation than meat, due to the high degree of unsaturation in lipids and also to the high concentrations of metals in seafood (Secci and Parisi, 2016). Rainbow trout (*Oncorhynchus mykiss*) is a fatty fish species, and quality deterioration of fatty fish species is primarily caused by microorganisms and lipid oxidation (Rezaei and Hosseini, 2008).

Oxidation typically includes the reaction of oxygen with the double bonds of fatty acids. Lipid oxidation is one of the major causes limiting the quality and acceptability of meat and meat products. This process creates drip losses, loss of nutrient value, off-flavor developments and production of toxic compounds. Consumers are less likely to consume meat and meat products that have these quality problems. Therefore, it is important for food processors to control lipid oxidation to maintain both product quality and wholesomeness (Ghaly *et al*., 2010).

Lipid oxidation consists of three steps initiation, propagation and termination (Khayat and Schwall, 1983; Erkan and Özden, 2006). The initiation step involves the formation of free radicals which react with oxygen to form peroxyl radicals. In the propagation step, the peroxyl reacts with other lipid molecules to form new free radicals and hydroperoxides (Fraser and Sumar, 1998). Finally, in the termination step, free radicals interact to form nonradical products (Ghaly *et al*., 2010).

Peroxide value (PV) is the most common measurable parameter of the fatty acid hydroperoxidation which is the primary product of lipid oxidation. Secondary products of lipid oxidation are to measure the Thiobarbituric value (TBA) (Melton, 1983). The oxidation process can be influenced by both intrinsic and extrinsic factors, such as the concentration of prooxidants, the fatty acid composition, myoglobin, pH, temperature, enzymes, ionic strength, and oxygen consumption (Andreo *et al*., 2003). In addition, total volatile basic nitrogen (TVB-N), trimethylamine (TMA), and formation of biogenic amines have been used as freshness indicators (Özogul *et al*., 2004; Rezaei and Hosseini, 2008).

To retard or prevent oxidative deterioration, antioxidants, especially synthetic ones, have been widely employed. However, they may cause the negative side effect for consumers (Lim *et al*., 2011). Several studies have reported the use of various antioxidants to control lipid oxidation in fish systems. Hence, there is a tendency towards the use of natural antioxidants, particularly from plant origin to replace those of synthetic origin (Zhang *et al*., 2010; Takeungwongtrakul and Benjakul, 2014).

Chitosan has a potential to use as food packaging, especially as edible films and coatings because of its unique property of increased viscosity upon hydration. A

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number of functional properties including antioxidant, antimicrobial, and oxygen barrier properties have been reported for chitosan film (Sathivel, 2005). Pectin has been used as emulsifier and filler in food industry. Many coating materials have been tested in an attempt to maintain quality and prolong shelf life of meat products. Among them, essential oils, which are oily aromatic liquids extracted from aromatic vegetable plants and widely used in foods (Burt, 2004). Essential oils are composed of complex mixtures of monoterpenes, biogenetically related phenols, and sesquiterpenes (Isman *et al*., 2007). Additionally, essential oils have been reported to possess antioxidative activity and reduce oxidation (Singh *et al*., 2012).

In our previous work (Tabatabaei Moradi *et al*., 2015), we established and studied the antibacterial activity of lemon and *M. piperita* essential oils. The aim of the present work was to compare, for the first time, the antioxidation property of different percentages of these **e**ssential oils obtained via hydrodistillation method on rainbow trout fillet during 16-day refrigerated at 4±1˚C storage.

Materials and methods

Materials

Two live rainbow trout was purchased from the seafood market and transported to the laboratory in polystyrene boxes at low temperature. It was then gutted, washed, and cut into average weight 100±10 g slices. The sour lemon was obtained in spring from Tehran market. The *M. piperita* was obtained from Iranian Institute of medicinal plants**,** Karaj, Iran. Chitosan solution was prepared with 2 g chitosan (medium MW, deacetylated chitin, 448877; 95e98%; Sigma-Aldrich) and 1 g acetic acid (Sigma-Aldrich) in 100 ml distilled water. It was stirred for 3 h at room temperature to achieve homogenous solution. Pectin solution (obtained from citrus peel, 2g $100g^{-1}$, galacturonic acid ≥74.0% (anhydrous basis), P8471, Sigma-Aldrich) was added in 70°C distilled water and stirred for 1 h to achieve complete dispersion of Pectin, to applied in order to crosslink the polymers, calcium chloride (2g $100g^{-1}$, C5670, Sigma-Aldrich) was made using distilled water.

Extraction procedure

The amount of 200 g dried *M. piperita* leaves were ground and mixed three times and its weight distilled water delivered in a Clevenger type distillation apparatus for hydrodistillation in 5 h. The essential oil was dewatered over anhydrous sodium sulphate and collected in colored glass vial. In the next step, 300 g lemon was added in the Clevenger type distillation for 2 h and the product was collected in a similar way of the previous method (Mahato *et al*., 2017).

Analysis of essential oil components

Components of the extracts were analyzed using a HP-6890 GC-MS system (Hewllet Pachard, USA). The compounds of lemon and *M. piperita* essential oil were separated on 60 m × 0.2 mm × 0.25 μm HP-1MS column (methyl silicon-cross link, Hewllet Pachard, HP 5973, USA). Helium was used as the carrier gas, the flow rate was 1.0 ml min^{-1}; the column temperature was increased from 160 ºC to 230 ºC at a rate of 7 ºC min^{-1}; injector temperature was 250 ºC; injection volume

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was 1 µl; and transfer temperature was 280 ºC. MS parameters were as follows: EI mode, with ionization voltage 70 eV, ion source temperature, 150 ºC; mass analyzer temperature, 230 ºC; scan range. Identification of constituents was carried out with kovats index and retention times of standard substances by comparison of mass spectra with the data given in the literature (Adams, 2007).

Sample preparation

Preparation and coating of the fish samples were carried out as follows (Tabatabaei Moradi *et al*., 2015). Briefly, six samples of the rainbow trout fillet in average weight of 100±10 g consisting of one lot control (uncoated) and five lots coated were prepared (Table 1)**.** Slices fillet of rainbow trout were dipped into each coating solution (Table 1) for 2 min and the excess coating was allowed to drip off for 2 extra minutes. The control sample was only dipped into sterile distilled water for 2 min. After 8 min of drying at room temperature, the coated samples were placed into plastic containers polyethylene lid, and stored at 4±1˚C for 16-day.

Table 1: Preparation of layers on rainbow trout fillet.

Sample	Layers
Control	non-coated
CP	Calcium chloride +chitosan + pectin
LEO(0.5%)	Calcium chloride +chitosan + pectin+0.5% lemon
LEO(1%)	Calcium chloride +chitosan + pectin+1% lemon
PEO (0.5%)	Calcium chloride +chitosan + pectin+0.5% *Mentha piperita*
PEO(1%)	Calcium chloride +chitosan + pectin+1% *Mentha piperita*

Chemical analysis

Protein, fat, moisture and ash contents of rainbow trout fillet samples were determined according to AOAC (1990) (Chemists and Helrich, 1990). Changes in pH values were recorded (pH meter Metrohm, type 827) at initial day intervals up to day 16 of storage at 4±1˚C.

Assessment of lipid oxidation

The evaluation of lipid oxidation was achieved with three different indices, the peroxide value (PV) measurement (Egan *et al*., 1981), the 2-thiobarbituric acid (TBA) assay (Goulas and Kontominas, 2005) and free fatty acid**s** (FFA) (Egan *et al*., 1981) to assess the LEO and PEO essential oil on oxidation of fish meat in 16-day storage at 4±1˚C. The meat deterioration was evaluated with total volatile basic nitrogen (TVB-N).

Sensory evaluation

The sensory quality of rainbow trout control and coated fillets were evaluated by seven members trained panel. Panelists were scored for sensory characteristics such as colour, odour, flavour, general acceptability and texture, using a ten-point hedonic scale (1 as extremely dislike to 10 as extremely like). The fish samples were considered to be acceptable for human consumption until the sensory score reached 6 (Goulas and Kontominas, 2007).

Statistical analysis

All measurements were replicated three times and mean values±standard

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deviations were obtained for each case. Analysis of variance (ANOVA), Duncan multiple range test, least significant difference (p<0.05), were performed to evaluate the significance of differences among mean values, using the SPSS 20.0 for windows.

Results

Composition of extracted essential oil

The composition of the lemon and *M. piperita* essential oils were detected by GC-MS and presented in Fig. 1 a and b. The most abundant compounds found were limonene (28.7%), β-pinene (14.26%) and E-citral (8.42%) for the lemon and cyclohexanol (22.34%), methyl acetate (13.34%), 1-menthone (8.12%) for the *M. piperita*. Other identified compounds were less predominant in lemon and *M. piperita* essential oils.

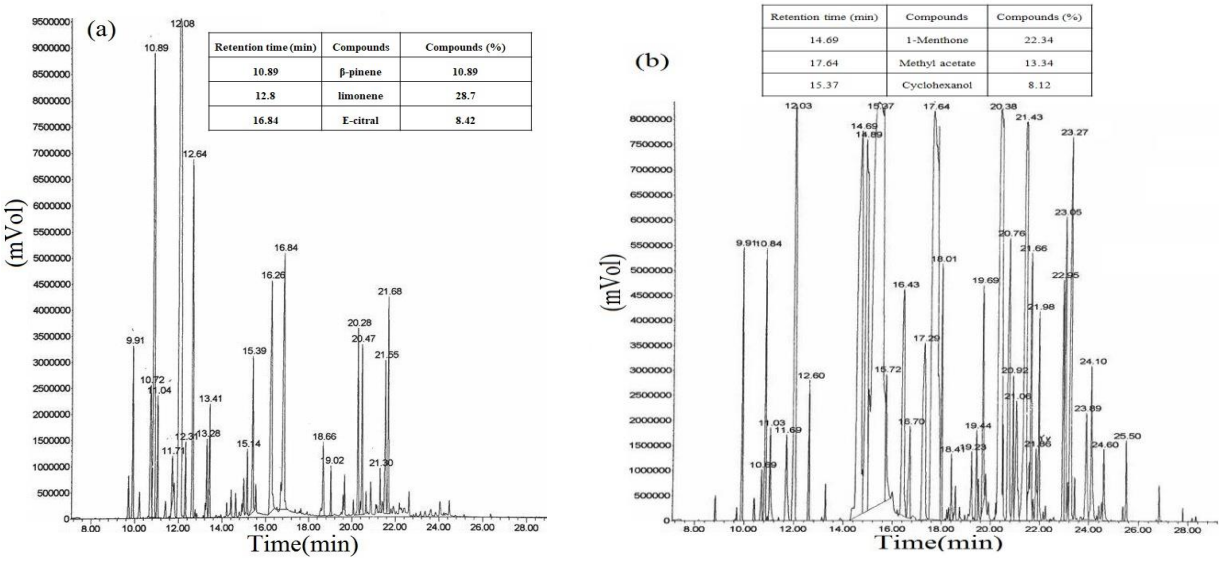


Figure 1: GC chromatograms obtained from analysis of number corresponds to β-pinene (10.89 min), limonene (12.8 min), E-citral (16.84 min) for lemon essential oil (b) and cyclohexanol (15.37 min), methyl acetate (17.64 min), 1-menthone (14.69 min) for *Mentha piperita* essential oil.

Chemical properties

Chemical analysis of rainbow trout fillet displayed a protein content of 19.1±0.2%, fat 5.3±0.2%, moisture 68.2±0.3% and ash 1.21±1%. Several researchers reported different amounts of chemicals in fish proximate compositions, especially in fat which might be related to feeding, fish season, sex differences, size of fish and environment conditions .

The initial pH of control sample was measured 6.45 and increased to 6.95 at the end of 16 days storage at 4±1˚C, but pH values were significantly different (p<0.05) than the control and decreased to 6.09, 5.44, 5.67, 5.17, and 5.43 for CP, LEO (5%), LEO (1%), PEO (5%) and PEO (1%), respectively. The pH values of the samples which were covered with LEO and PEO were significantly different (p<0.05) than those of the control. The lower pH prevents activity of spoilage bacteria and endogenous enzymes, and so leads to the extended preservation of fish.

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Lipid oxidation

Peroxide value

The content of primary lipid oxidation products was measured as peroxide value (PV) .The initial PV (meq peroxide kg^{-1} fish sample) in the control sample was analyzed and ranged from 0.9 to 20.3 after 16 days storage at 4±1˚C. After 4 days, no obvious difference has been observed throughout the storage in samples which were covered with LEO and PEO (0.5 and 1%). By the end of the storage time, significant differences (p<0.05) were observed in the PV between the control (20.03) and each of LEO (1%) and PEO (1%) covered samples, which exhibited lower values of 7.43 and 5.15, respectively (Fig. 2). PV of the sample covered with PEO (0.5 and 1%) was also significantly lower than the sample covered by LEO (0.5 and 1%). Storage time has a significant effect on the PV for both the control and covered samples. All samples were below the proposed acceptable level of 10–21 meq peroxide kg^{-1} fish fat .

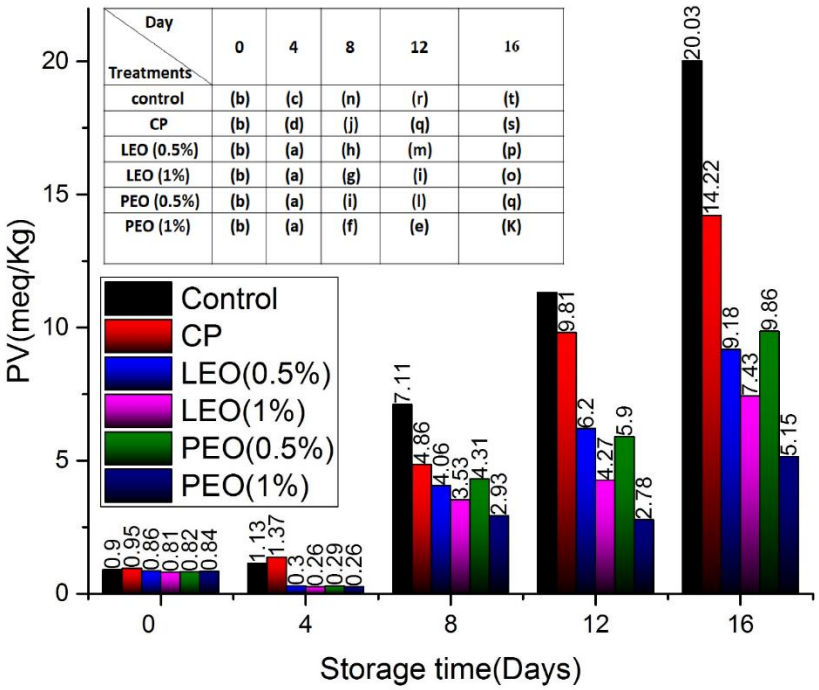


Figure 2: Changes in PV values during storage time. Different small letters in the table represent significant difference at p<0.05.

2-Thiobarbituric acid

2-Thiobarbituric acid (TBA) value has been widely used for the assessment of degree of lipid oxidation and the presence of TBA reactive substances (Shahidi, 1994). Changes of TBA value are shown in Fig. 3. In this study, the initial TBA value of control sample was 0.13 mg kg^{-1} and the value exceeded 0.77 mg kg^{-1} on day 16 and 0.3 and 0.23 of storage for LEO and PEO (1%), respectively.

The value of LEO (0.5 and 1%) and PEO (0.5 and 1%) were significantly (p<0.05) lower than the control sample throughout the storage period, indicating that the LEO and PEO essential oil coating effectively inhibited the lipid oxidation. The results show that covering fish meat

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with LEO and PEO could control lipid oxidation efficiently.

Compared to the control and CP, the TBA values of LEO (1%) and PEO (1%) were lower due to the presence LEO and PEO essential oil and after 16 days, PEO (1%) had the best effect.

Day / Treatments	0	4	8	12	16
control	(abcde)	(cdefg)	(ij)	(k)	(l)
CP	(abc)	(abcdef)	(fghi)	(hi)	(jk)
LEO (0.5%)	(ab)	(abcde)	(cdefg)	(ghi)	(ii)
LEO (1%)	(a)	(abc)	(abcdef)	(efgh)	(ghi)
PEO (0.5%)	(ab)	(abcd)	(fghi)	(bcdefg)	(ii)
PEO (1%)	(a)	(abc)	(abcde)	(bcdefg)	(defg)

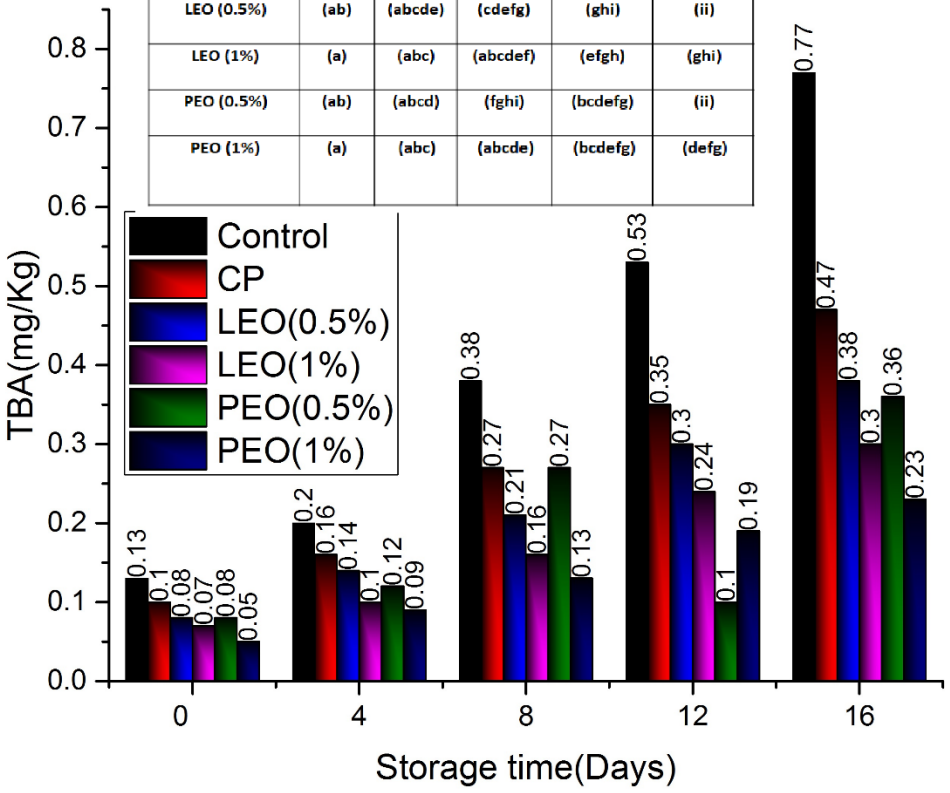


Figure 3: Changes in TBA values during storage time. Different small letters in the table represent significant difference at $p<0.05$.

Free fatty acids

Generally, FFA is the hydrolysis products of fat and oil oxidation during storage or processing new products. FFA is one of the main feature linked with the quality and commercial value of oils and fats .

Changes in FFA values of control and different covering samples are given in Fig. 4. An increase in FFA value was observed in all of the samples during 16 days of storage. The increase rate was higher ($p<0.05$) in the control than the samples covered with CP, LEO (0.5 and 1%) and PEO (0.5 and 1%). The significant difference ($p<0.05$) was observed in 1% PEO compare to LEO group.

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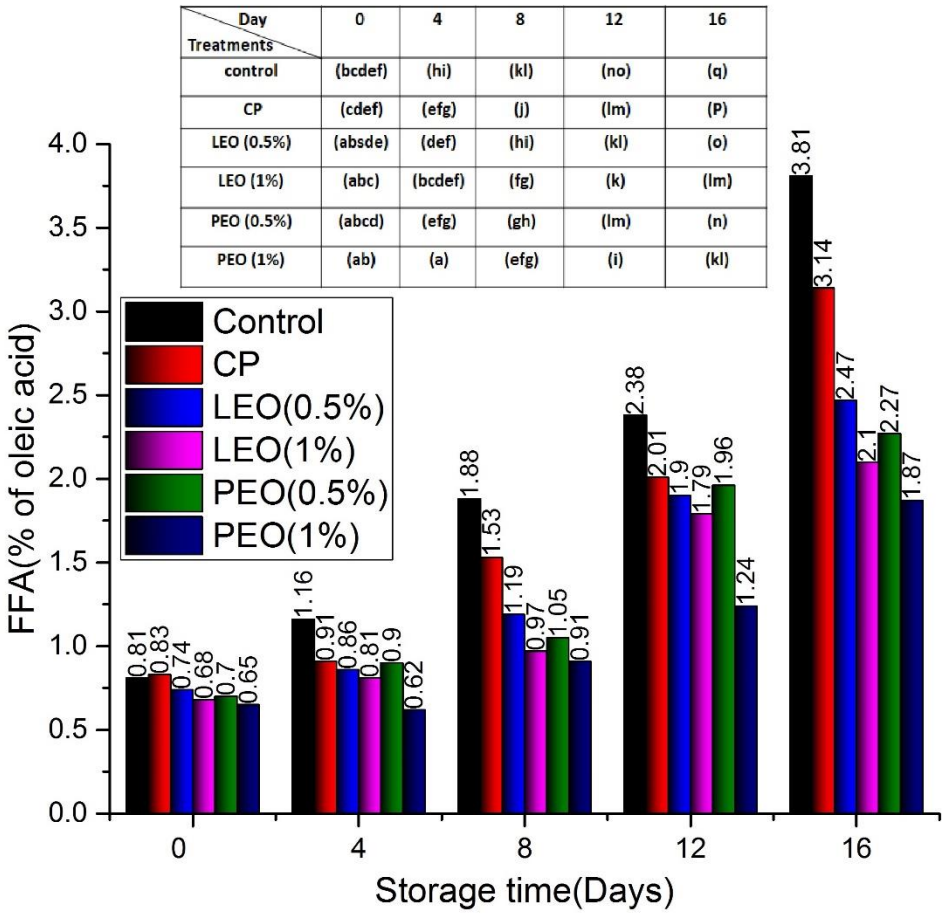


Figure 4: Changes in FFA values during storage time. Different small letters in the table represent significant difference at $p<0.05$.

Lipid oxidation, defined as the oxidative deterioration of unsaturated fatty acids, is a free radical-mediated phenomenon. It can give rise to several degenerative conditions and can cause the qualitative deterioration of muscle foods flavor and odor effects (Bhandari, 2012). While, FFA increased after 16 days storage but PEO (1%) had the lowest FFA content compare with another covering samples.

Total volatile basic nitrogen

Fig. 5 shows the effect of covering with LEO and PEO on TVB-N production in the fish samples stored at 4±1˚C in 16-day. TVB-N, which is mainly composed of ammonia and primary, secondary and tertiary amines, is widely used as an indicator of meat deterioration. The increase in TBA-N was retarded in the presence of LEO and PEO essential oil. Amongst all samples, the control sample showed the highest TBA-N in comparison with the other samples throughout 16 days storage ($p<0.05$). The increase in TBA-N value of lipids indicated the formation of the secondary lipid oxidation products.

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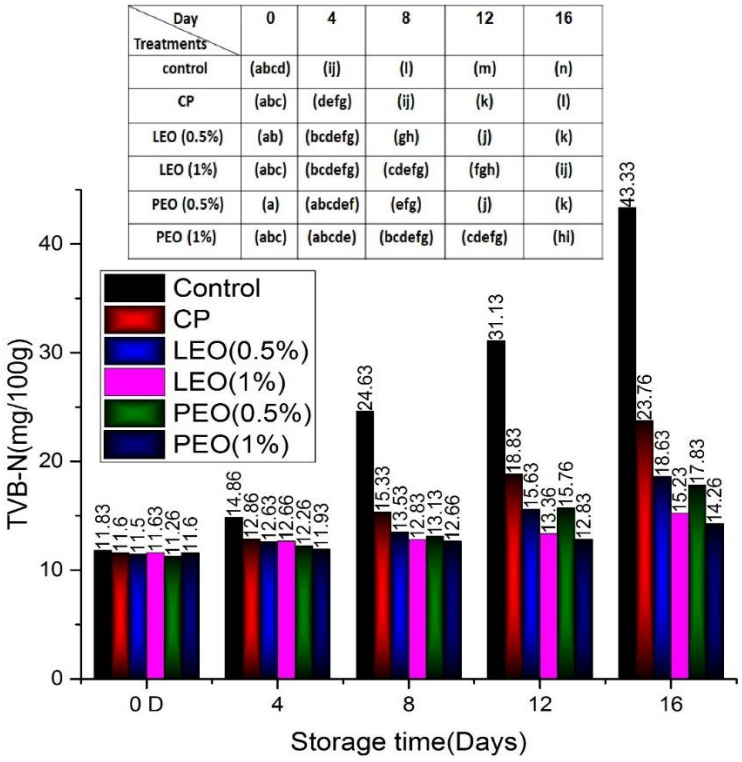


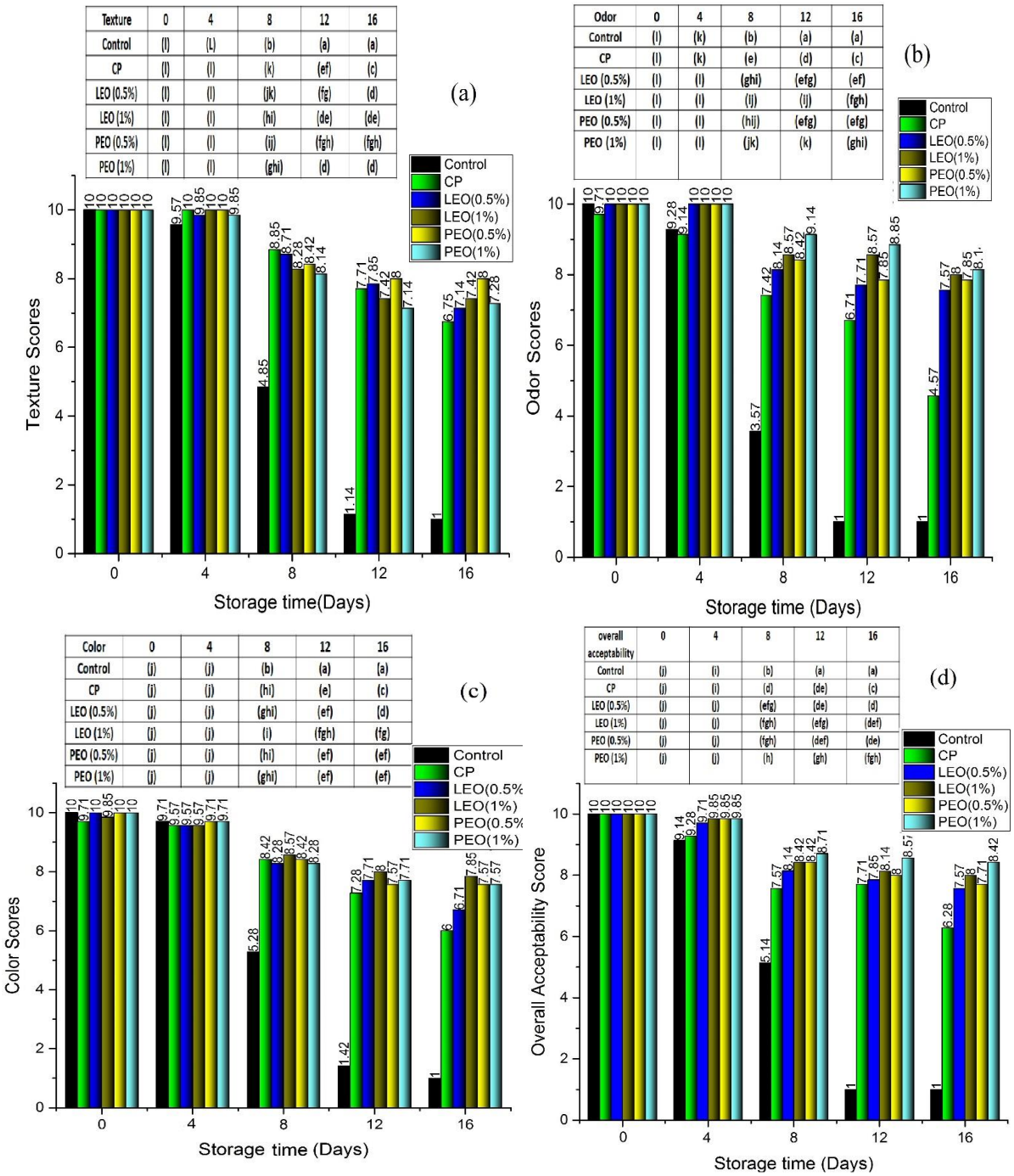
Figure 5: Changes in TVB-N values during storage time. Different small letters in the table represent significant difference at $p<0.05$.

Sensory evaluation

The fish samples were considered to be acceptable for human consumption until the sensory score reached 6. The changes in attribute scores of sensory evaluations of control and covered rainbow trout fillets with LEO and PEO (0, 0.5 and 1%) during 16 days of refrigerated storage at 4±1˚C are shown in Fig. 6. The samples which were covered with LEO (0.5 and 1%) and PEO (0.5 and 1%) showed sensory scores above the rejection limit (6) throughout the 16 days storage. The odor and color scores (Fig. 6(b) and 6(c)) of fish meat covered with LEO (0.5 and 1%) and PEO (0.5 and 1%) were significantly higher ($p<0.05$) than the control and CP throughout the 16 days storage. The score of texture and overall acceptability of fish meat (Fig. 6(a) and 6(d)) covered with CP, LEO (0.5 and 1%) and PEO (0.5 and 1%), compared to the control, were significantly higher ($p<0.05$) throughout the 16 days storage. Due to high lipid oxidation, the control samples (uncoated) of rainbow trout fillet showed spoilage after 8-day storage. The antioxidant effects of samples coated with LEO (0.5 and 1%) and PEO (0.5 and 1%) have been shown to minimize oxidative effects on texture, odor, color and acceptability of the fish in 16 days.

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Texture	0	4	8	12	16
Control	(l)	(L)	(b)	(a)	(a)
CP	(l)	(l)	(k)	(ef)	(c)
LEO (0.5%)	(l)	(l)	(jk)	(fg)	(d)
LEO (1%)	(l)	(l)	(hi)	(de)	(de)
PEO (0.5%)	(l)	(l)	(ij)	(fgh)	(fgh)
PEO (1%)	(l)	(l)	(ghi)	(d)	(d)

Odor	0	4	8	12	16
Control	(l)	(k)	(b)	(a)	(a)
CP	(l)	(k)	(e)	(d)	(c)
LEO (0.5%)	(l)	(l)	(ghi)	(efg)	(ef)
LEO (1%)	(l)	(l)	(ij)	(ij)	(fgh)
PEO (0.5%)	(l)	(l)	(hij)	(efg)	(efg)
PEO (1%)	(l)	(l)	(jk)	(k)	(ghi)

Color	0	4	8	12	16
Control	(j)	(j)	(b)	(a)	(a)
CP	(j)	(j)	(hi)	(e)	(c)
LEO (0.5%)	(j)	(j)	(ghi)	(ef)	(d)
LEO (1%)	(j)	(j)	(i)	(fgh)	(fg)
PEO (0.5%)	(j)	(j)	(hi)	(ef)	(ef)
PEO (1%)	(j)	(j)	(ghi)	(ef)	(ef)

overall acceptability	0	4	8	12	16
Control	(j)	(i)	(b)	(a)	(a)
CP	(j)	(i)	(d)	(de)	(c)
LEO (0.5%)	(j)	(j)	(efg)	(de)	(d)
LEO (1%)	(j)	(j)	(fgh)	(efg)	(def)
PEO (0.5%)	(j)	(j)	(fgh)	(def)	(de)
PEO (1%)	(j)	(j)	(h)	(gh)	(fgh)

Figure 6: Sensory evaluation of rainbow meat covered with LEO (0.5 and 1%) and PEO (0.5 and 1%), texture (a), odor (b), color (c) and overall acceptability (d). Different small letters in the tables represent significant difference at $p<0.05$.

In the present study, it could be concluded that rainbow trout fillet coating with LEO and PEO (0.5 and 1%) stored efficiently according to the quality compare to the control. According to the results of pH, PV, TBA, FFA, TVB-N and overall sensory scores obtained, the samples covered with LEO and PEO successfully inhibited the lipid oxidation during 16 days of refrigerated storage at 4±1˚C. Rainbow trout fillet coating with PEO (1%) showed better results to increase TVB-N (14.26 mg N $100g^{-1}$) and FFA (1.87%) compare with rainbow trout

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stored on ice for 16 dayes. (TVB-N 14.26 mg N $100g^{-1}$ and FFA 1.87%)fillet multilayered edible coating with an antimicrobial LEO and PEO (0.5 and 1%) essential oil can properly delay the growth of spoilage microorganisms and prolong the shelf life of meat products .

Discussion

In this study, the main objective was to assess the effectiveness of a multilayered antimicrobial edible coating using the Layer-by-Layer (LbL) technique to extend the shelf life of rainbow troat fillets in refrigerated storage.

Limonene is the main volatile component of citrus fruits including, other than lemon, orange, bergamot and mandarin (Fisher and Phillips, 2006; Viuda-Martos *et al*., 2008; Espina *et al*., 2011). However, it has been demonstrated that the antimicrobial activity of citrus EO is not related to Limonene, but instead it seems to be due to the presence of other constituents such as oxygenated monoterpenes (Chutia *et al*., 2009).

The initial pH in this study was 6.45, which was sparingly lower than that reported by Volpe *et al*. (2015).

According to their study performed in Italy the effect of carrageenan based biocomposites on shelf life of rainbow trout fillets during refrigerated storage were investigated which show similar result for pH. In all fish samples, the values of pH decreased initially and then increased. Similar observations were made by Alasalvar *et al*. (2001) and Manju *et al*. (2007). Arashisar *et al*. (2004) demonstrate that the initial decrease of pH value may be related to the dissociation of carbonic acid in the fish samples for fish fillets. Chaijan *et al*. (2006) believed that increased volatile bases that were produced by either endogenous or microbial enzymes led to an increase in pH [36]. Masniyom *et al*. (2002) reported that the decomposition of nitrogenous compounds caused an increase in pH in fish flesh.

2-Thiobarbituric acid (TBA) is an index of lipid oxidation. The results showed that TBA value of all treatments increased continuously during storage. This observation was similar to the results from Kamil *et al*. (2002), Manju *et al*. (2007), Fan *et al*. (2009) and Lu *et al*. (2010). Increase in TBA value may be attributed to the partial dehydration of fish and the increased oxidation of unsaturated fatty acids. According to Connell (1990), a TBA value of 2 mg MDA/kg was regarded as the limit beyond which the fish will normally develop an objectionable odour and taste. Lipid oxidation can be initiated and speed up with various mechanisms including the production of singlet oxygen, non-enzymatic and enzymatic generation of free radicals and active oxygen (Song *et al*., 2011). The fish surface covered with CP layer may has been resistant to oxygen diffusion, thus may has retarded lipid oxidation in compare to control sample (Butler *et al*., 1996).

TVB-N value was found to grow up in all samples during storage. This excess is related to the activity of spoilage bacteria and endogenous enzymes (Kyrana *et al*., 1997; Vareltzis *et al*., 1997). TVB-N value of all samples showed a slow increase in the early storage, but a marked increase was observed after day 12. This trend agrees with previous related research

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concerning other fish species (Aubourg, 2001; Quitral *et al*., 2009).

The data also showed that in all samples covered with LEO (1%) and PEO (1%) essential oil, the increasing rate of TBA-N was approximately constant within the 16 day-storage but significantly different with LEO (0.5%) and PEO (0.5%) (p<0.05). Thus, it can be concluded that essential oils, particularly PEO (1%), showed an effectiveness in the prevention of the oxidation of lipids.

The sensory qualities of fish samples were evaluated in terms of colour, odour, texture, and overall acceptability. The results were in accordance with Lu *et al*. (2010) who found that the shelf life of untreated northern rainbow troat fillets was 7 days according to sensory score, and the fish with LEO (0.5 and 1%) and PEO (0.5 and 1%) coating were still considered to be acceptable during this storage period.

In the present study, it could be concluded that rainbow trout fillet coating with LEO and PEO (0.5 and 1%) better maintained the quality in compare to control. According to the results of pH, PV, TBA, FFA, TVB-N and overall sensory scores obtained, the samples covered with LEO and PEO successfully inhibited the lipid oxidation in during 16-days of refrigerated storage at 4±1˚C. Rainbow trout fillet coating with PEO (1%) shows better results to increase TVB-N (14.26 mgN $100g^{-1}$) and FFA (1.87%) in compare to rainbow trout stored in ice for 16-dayes. (TVB-N 14.26 mgN $100g^{-1}$ and FFA 1.87%) (Rezaei and Hosseini, 2008). Rainbow trout fillet multilayered edible coating with an antimicrobial LEO and PEO (0.5 and 1%) essential oil can properly delay the growth of spoilage microorganisms and prolong the shelf life of meat products (Tabatabaei Moradi *et al*., 2015).

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