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Effect of packaging with nano-composite clay/LDPE film on the quality of rainbow trout (*Oncorhynchus mykiss*) fillet at refrigerated storage

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Abstract

In recent years, application of nanotechnology in the food packaging industry has become more widespread and is progressively being commercialized. Adding nano-materials improves polymers` barrier properties for gases such as O_2 and Co_2, and increases UV rays barrier, mechanical strength, stiffness, stability, and heat resistance of the base polymer. In this study, low density polyethylene film (LDPE) with 5 wt % nanoparticles of clay was used as the experimental treatment and low density polyethylene film as control treatment. These films were used for packaging of rainbow trout fillets and keeping them in the refrigerator. Then to investigate the quality of packed fillets, samples were taken in days 0, 5, 10, 15, 20 and 25, and were evaluated using chemical (pH, PV, TBA, TVB-N), microbial (TVC, PTC, LAB, EBC and H_2S producing bacteria) and sensory tests. Based on the results, significant differences (p<0.05) were observed between control and experimental treatments in different days. The sensory attributes of rainbow trout fillets correlated well with the microbiological analyses (r=0.91). With regards to sensory scores and the microbiological analysis, the shelf-life of rainbow trout fillets has been determined in the control and experimental treatments, 13 to 15 and 18 to 20 days, respectively.

Keywords: Rainbow trout, Low density polyethylene (LDPE), Nano- clay

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Introduction

Rainbow trout (*Oncorhynchus mykiss*) belongs to the family salmonidae, one of the main species with high commercial value and much appreciated by Iranian consumers. It is sold as either whole fresh fish or in fillet form (Etemadi *et al*., 2013). The quality of fresh fish is a major concern to the consumers and industry. Fish is extremely perishable and the shelf life of such products is limited in the presence of normal air by the chemical effect of atmospheric oxygen and the growth of aerobic spoilage microorganisms. It is generally accepted that the environment can influence the microflora associated with the skin, gills and intestine of finfish (Horsley, 1973). Rainbow trout is a fatty fish containing high levels of polyunsaturated fatty acids (PUFA), which are very sensitive to lipid oxidation, and therefore limits its shelf life (Vicetti *et al*., 2004; Viscidi *et al*., 2004). Because of the benefits of these fatty acids for health, consumption of this fish is remarkably recommended (Hosseini *et al*., 2010)., Spoilage of the product is determined in numerous ways, including sensory, biochemical, physical and microbiological (Gill, 1992; Gram and Huss, 1996). Bacterial spoilage happens in the fish stored in the refrigerator, under aerobic conditions by psychotropic gram-negative microorganisms such as Pseudomonas, Altermonnas, Shewanella and various species of Flavobacterium (Hubbs, 1991). Actions to prevent or postpone the deterioration of fish and its products have been reported, including cooling the product immediately after the catch, keeping in ice, packaging in vacuum and modified atmosphere, gamma and UV irradiation (Savvaidis *et al*., 2002), The main role of packaging, in addition to marketing and giving information to consumers (Gomez-Guillen *et al*., 2009), preserving the quality and safety of food, is controlling safe transfer of material among food, packaging and the atmosphere (preventing the migration process), as well as good protection against light, UV ray and mechanical damage (Baldwin and Hagenmaier, 2012). The polymers used in this research have special characteristics and potential to be used in commercial products. The polyethylene (PE) is a flexible semi-crystalline polymer whose properties are influenced by the relative amount of amorphous and crystalline phases (Jokar, 2010). One of the most common polymers is the low-density polyethylene (LDPE), because it possesses many desirable qualities such as transparency, water vapor impermeability, heat seal ability, chemical parts and is fairly economical. But the organic vapors and oxygen and carbon dioxide permeability are high and LDPE has poor grease barrier property (Coutinho *et al.*, 2003). Adding clay nanoparticles (1-2 nm) to low density polyethylene (LDPE) improved the physical and mechanical properties as well as the barrier feature of film to gases such as CO_2, O_2 and N_2 (Alexandre and Dubios, 2000). Translocation of nano-particles between polymer chains and creation of strong interactions lead in strengthening the

polymer network and improvement in the mechanical properties, hindrance to gases (oxygen and carbon dioxide), reduced permeability to water vapor and increase in heat resistance (Arora and Padua, 2009). Thus the objective of the present work was to determine the effect of packaging with nano-composite clay/LDPE film in combination with storing in refrigerator (4±2 °C) by evaluating certain chemical (pH, PV, TBA, TVB-N), microbiological (TVC, PTC, LAB, EBC and H_2S producing bacteria) and sensory parameters.

Materials and methods

Clay/LDPE nano-composite film production:

To produce composite low density polyethylene film and 5 wt % nanoparticles of clay, 3 wt % of polyethylene maleic anhydride (PEMA, Gran Kane, Iran), 5 wt % of Ethylene vinyl alcohol (EVA, Grade 910, EVA Chemi, Iran), 5 wt % of Montmorillonite (MMT) Na^+ (Sigma Aldrich), 10 wt % of linear low density polyethylene (LLDPE, Grade 0209, Arak Petrochemical, Iran) and 77 wt % of low density polyethylene (LDPE, Grade 0075, Bandar Imam Petrochemical, Iran) were mixed and stirred until complete coverage of nano-particles of clay on granules was reached. This continued to the final mixture in a twin screw extruder (Brabender, l/d=40, h=80cm, made in Germany). The reaction process was conducted at temperature of 150 °C and rotation of 80 rpm; then, nano-composite granules produced by single-screw extruder (Brabender, l/d=26, h=52cm, made in Germany); meanwhile, films with thickness of 40±2 μm were produced. This was done in Iran Polymer and Petrochemical Institute.

Preparation of fish

Eight kilograms of alive rainbow trout (average weight 300±25 g, in autumn season) were purchased from market and transferred to the aquatic processing research center (Anzali, Iran) using Styrofoam container having ice by ratio of 2 to 1 (fish to ice). After washing and sterilizing tables and tools, fish were rinsed with cold water and then changed to fillet. Each fillet was put into low density polyethylene bags as control treatment and low density polyethylene with 5 wt % clay nanoparticles bags as experimental treatment. After placing in the refrigerator at 4±2 °C, in day intervals of zero, 5, 10, 15, 20 and 25, they were sampled and studied in terms of the quality indicators, including chemical analysis (pH, TVB-N, PV, TBA), bacterial (TVC, PTC, LAB, EBC, H_2S producing bacteria) and sensory evaluation.

Chemical analysis

Lipid, protein, ash and moisture content of samples were determined using (AOAC, 2005) method. The pH value was determined using digital pH-meter (Switzerland Az86p3). The amount of PV, expressed as mEq O_2 kg^{-1} oil, was determined according to the Egan *et al*., (1997) method. Thiobarbituric acid (TBA) (malondialdehyde mg kg^{-1} fish

flesh) was determined according to the Kirk and Sawyer (1991) method. The TVB-N content of rainbow trout was determined according to the method of Goudlas and Kontaminas (2005) and expressed as mg N $100g^{-1}$ flesh.

Microbiological analysis

Microbial assays include total viable count (TVC), psychrophilic total count (PTC), lactic acid bacteria (LAB), Enterobacteriaceae (EBC) and H_2S producing bacteria. For all microbiological counts, the skin from the anterior dorsal area was first washed with 70% alcohol and then aseptically removed using sterilized scalpels (Slattery, 1988). Then 10 g of flesh with both white and dark muscles were taken and transferred into 90 mL of 0.1% peptone water (Difco, 0118-17-0). From this dilution, other decimal dilutions were prepared. In this study, TVC and PTC were determined using plate count agar (PCA), according to the standard American Public Health Association method (APHA, 2001) through counting the colony forming units ($log_{10}CFU\ g^{-1}$) after incubating the plates at 30 °C for 48 h or 7–10 °C for 10 days, respectively. To count lactic acid bacteria, medium of De Man Rogasa and Sharpe (MRS) agar was applied, with 2 gas packs of type C inside of the anaerobic jars at 30°C for 48 hours. To count Enterobacteriaceae, medium of Violet Red Bile Glucose Agar (VRBGA) was used after 24 hours of incubation at 30 °C (ICMSF, 1978). Ultimately, for counting H_2S producing bacteria, especially *Shewanella putrefaciens*, the medium of Plate Iron Agar was applied at 20°C for four days (Gennari and Campanini, 1991). All of the microbial analyses were performed in triplicate on three subsamples of every replicates. All media were purchased from Oxide Inc (London, UK).

Sensory evaluation

Quality Index Method (QIM) was used for sensory evaluation of rainbow trout fillet. Factors like skin, color, smell and texture were evaluated by seven trained evaluators in two treatments, including common packaging with low density polyethylene film and other, common packing with nano-composite film clay/low density polyethylene. The scoring was from 0 to 10, as follows: the excellent quality from 0 to 1.5, good quality from 1.5 to 3, average quality from 3 to 5, and after 5 is unacceptable quality (Cristiana, 2013).

Statistical analysis

Statistical analysis of data obtained from the two treatments in three replications was done using SPSS 18 software. Differences between means ($p<0.05$) for the microbial and chemical results were analyzed using one-way analysis of variance (ANOVA) followed by Duncan's test. For sensory evaluation, a non-parametric ANOVA developed by Kruskal–Wallis was used.

Table 1: QIM-Scheme for sensory evaluation of rainbow trout fillet by Cristiana (2013).

Quality parameter	Description	Score
Skin appearance	Shiny and bright	0
	Slow density and shiny	1
	Opaque or Turbid	2
Color	Pink	0
	Pinkish or Yellowish	1
	Dark pink or yellow	2
Odor	Fresh fish and neutral	0
	Seaweeds	1
	Sour milk	2
	Ammonia or acetic	3
Texture	In rigor, Stiff or Horny	0
	Elasticity	1
	Soft	2
	Very soft or Pianissimo	3
Quality index (QI)		**0-10**

Results

Proximate composition of rainbow trout fillet (*O. mykiss*), are shown in Table 2, moisture, protein, lipid and ash contents of the samples were 72.16±3.54, 20.79±1.02, 5.99±0.87 and 1.04±0.2 %, respectively. The changes of pH in rainbow trout fillets during the storage at the refrigerator are shown in Figure 1a. The pH value in the first day of sampling in control and experimental treatments was 6.62 and 6.55, that in the continuation decreased and at end of the maintenance period, this amount reached to 6.79 and 6.63, respectively. However, the difference between the two treatments was not significant (p>0.05). Changes of peroxide value in rainbow trout fillet have increased in two control and experimental treatments during storage. Although the speed of increase of peroxide value in the control treatment was more than the experimental treatment, the peroxide value at the beginning of storage has been 0.51 and 0.63 mEq g O_2 Kg^{1-} Oil in control and experimental treatments, respectively, then increased and reached to 6.2 mEq gr O_2 Kg^{-1} Oil in control treatment on day 15, and in experimental treatment to 5.36 mEq gr O_2 kg^{-1} oil in day 20. Maximum permitted levels of peroxide in fish is 5 mEq gr O_2 kg^{-1} oil (Yanar, 2007). Significant difference (p<0.05) were also observed between treatments. Thiobarbituric acid (TBA) value is an index of lipid oxidation measuring malodialdehyde (MDA) content (Goulas and Kontominas, 2007). Fig 1c shows the amount of thiobarbituric acid which was 0.05 mg MDA Kg^{-1} of fish at the first day, and then increased in rainbow trout fillet during the storage at the refrigerator in both control and experimental treatments and on 25th day of storage, reached to 3.7 and 4.43 respectively, which showed no significant differences (p>0.05) between the two treatments. The Maximum Recommended Limit (MRL) for TBA in fish is 2 mg MDA Kg^{-1} of fish (Connell, 1990; Lakshanan, 2000). The amount of total volatile nitrogen bases (TVB-N), according to Fig. 1d, at the first day of sampling was 10.95 and

10.96 mg N $100g^{-1}$ for both treatments, respectively**.** TVB-N values increased during storage in the refrigerator, and it was 36.3 mg N $100g^{-1}$ on day 15 in the control treatment, and 37.1 mg N $100g^{-1}$ on day 20 in the experimental treatment. That represents the beginning of microbial spoilage. The changes in the microbial flora of rainbow trout samples during storage in varied time intervals are shown in Fig. 2. Total viable counts (TVC) are shown (Fig. 2a), in the rainbow trout fillet during storage in the refrigerator temperature of 4±2°C. The initial (day 0) TVC of fresh fish for control and experimental treatments were 2 and 1.5 log CFU g^{-1} muscle, but gradually increased to 7.03 log CFU g^{-1} in the control treatment on day 15 and to 7.23 log CFU g^{-1} in the experimental treatment on day 20. The Gram-negative Psychrotrophic bacteria are the major group of microorganisms responsible for spoilage of aerobically stored fresh fish at chilled temperatures (Gram *et al*., 1987; Gram and Huss, 1996). Figure 2b shows that the number of bacteria psychrophilic (PTC) has increased during storage in the refrigerator. The number of this bacteria in two treatments, control and experimental, on days 0, 15 and 20 was 1.7, 6.5 and 7.5 log CFU g^{-1} and 1.57, 4.72 and 6.56 log CFU g^{-1}, respectively. The difference was significant (p<0.05) between the two treatments. The number of lactic acid bacteria (LAB) and Enterobacteriaceae (EBC), based on Fig. 2c and 2d increased in control and experimental treatments during storage in the refrigerator temperature of 4±2 °C. The number of LAB at the end of storage, in control and experimental treatments, were 8 and 6.7 log CFU g^{-1}, respectively. The EBC in the initial sampling in control and experimental treatments was 0.99 and 0.86 log CFU g^{-1}, that reached to 9.6 and 8.45 log CFU g^{-1}, at the end of storage period in the control and experimental treatments respectively. Results showed significant differences (p<0.05) in each treatment between the first and last days of sampling; as well. The difference was not significant (p>0.05) between the two treatments, except in days 15 and 20. Based on Fig. 2e, the number of H_2S producing bacteria, especially *Shewanella putrefaciens*, at the first day were 1.52 and 1.46 log CFU g^{-1} in the control and experimental treatments, respectively, which increased significantly during the storage in both control and experimental treatments, and at the end, the number of these bacteria in both treatments were more than the permissible limit of 7 log CFU g^{-1}.

Table 2: approximate analysis of rainbow trout fillet.

Component	**Amount** (%)
Protein	20.79±1.02*
Lipid	5.99±0.87
Moisture	72.16±3.54
Ash	1.04±0.2

(Mean±Standard deviation*, N=3)

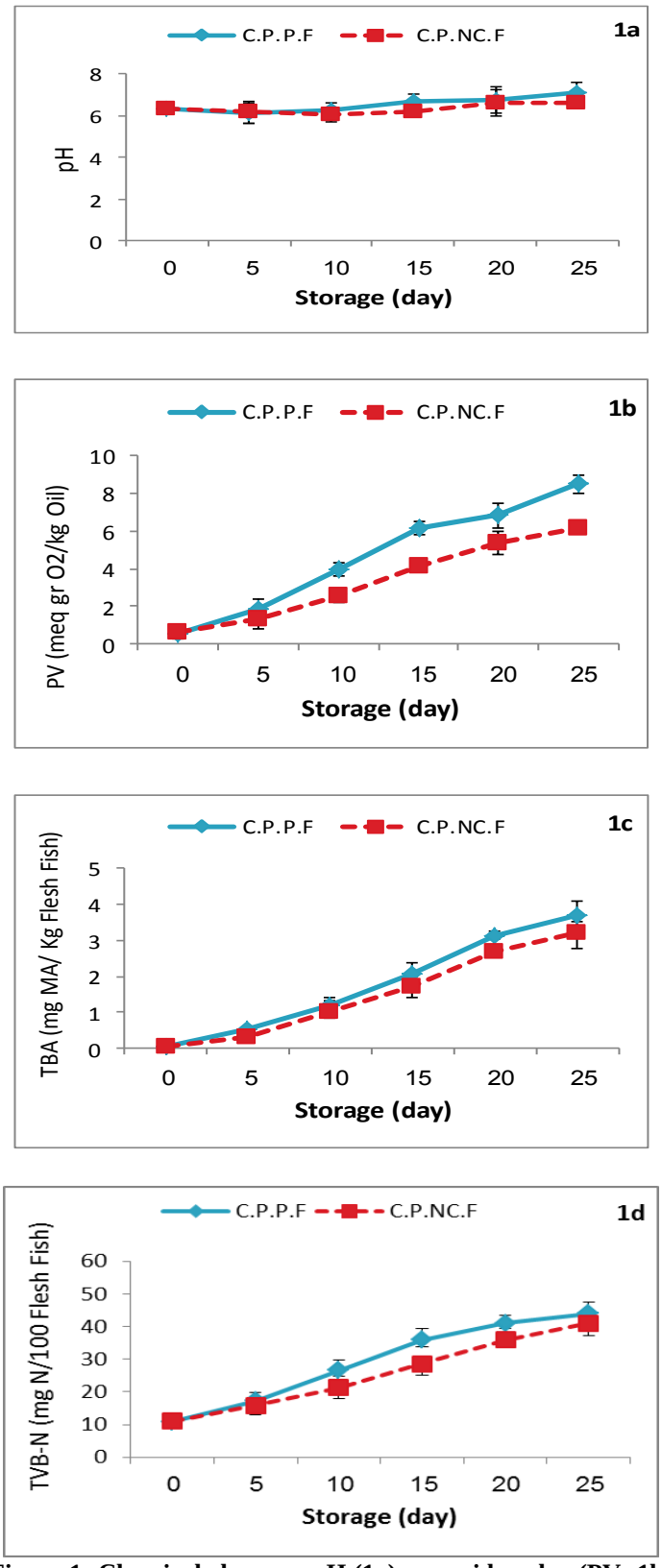

Figure 1: Chemical changes; pH (1a), peroxide value (PV; 1b), thiobarbitoric acid (TBA; 1c) and total volatile bases nitrogen (TVB-N; 1d) of rainbow trout fillet in the common packaging with low density polyethylene film (C.P.P.F) and common packaging with nano-composite film (C.P.NC.F) treatments during refrigerated storage.

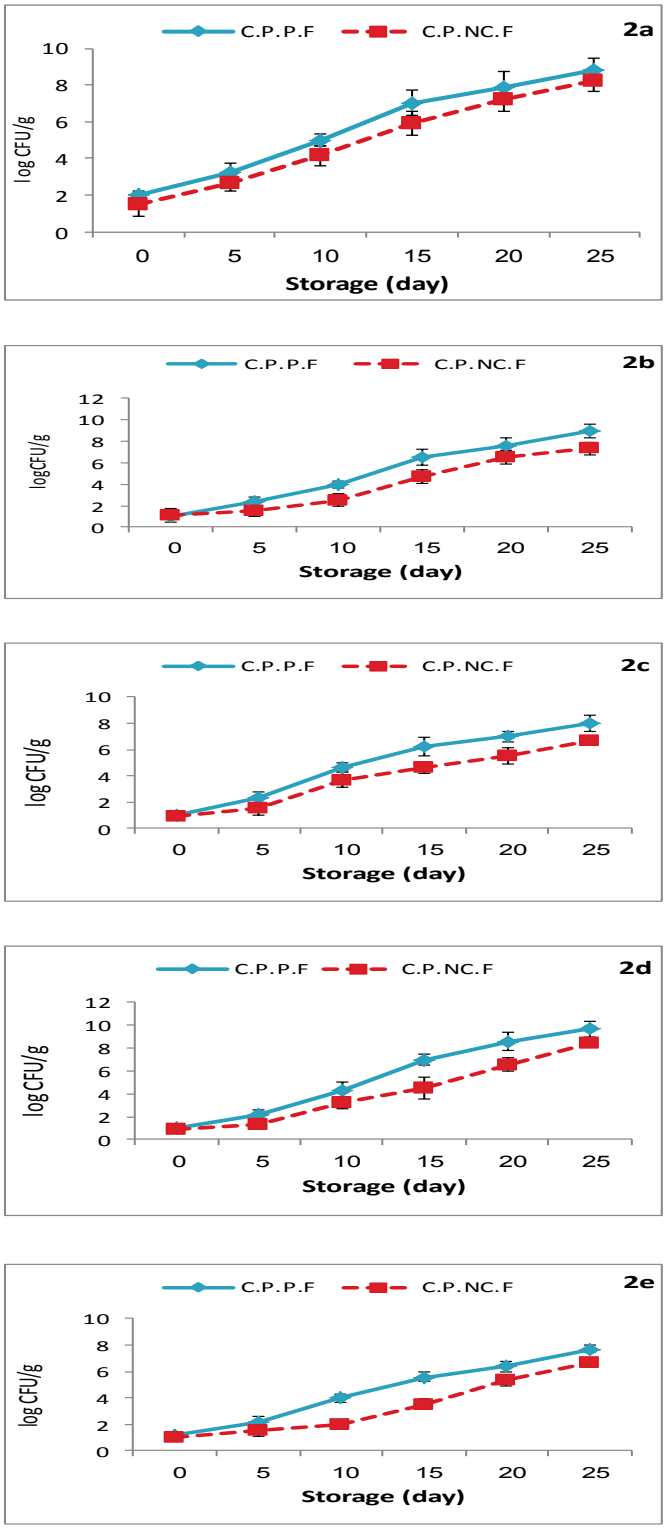

Figure 2: Microbiological changes; Total viable count (TVC, 2a), Psychrophilic total count (PTC, 2b), Lactic acid bacteria (LAB, 2c), Entrobactriaceae (EBC, 2d) and H_2S producing bacteria (2e) of rainbow trout fillet in the common packaging with low density polyethylene film (C.P.P.F) and common packaging with nano-composite film (C.P.NC.F) treatments during refrigerated storage.

Based on the results of Table 3, QIM-Scheme was used for sensory evaluation of rainbow trout fillet and factors such as skin color, texture and smell in two treatments, including common packaging with low-density polyethylene film (control) and common packaging with nano-composites film Clay/low density polyethylene (experimental). After 15 days, the amount of total factors for control treatment was 5.6, while in experimental treatment it was 7.8 on the 20^{th} days, was, that expresses the beginning of spoilage in treatments.

Table 3: Sensory evaluation of rainbow trout fillets in control and experimental treatments.

	Storage (day)					
Treatment	**0**	**5**	**10**	**15**	**20**	**25**
C.P.P.F	$0.4\pm0.02^{*Aa}$	1.6 ± 0.38^{Aab}	3.2 ± 1.2^{Ab}	5.6 ± 1.87^{Ac}	7.6 ± 2.58^{Acd}	9.4 ± 2.34^{Ad}
C.P.NC.F	0.2 ± 0.01^{Aa}	1.2 ± 0.56^{Aab}	2.6 ± 1.26^{Ab}	4.8 ± 1.68^{Ac}	7.8 ± 2.65^{Ad}	9.2 ± 2.45^{Ae}

(Mean±SD*, N=3) The small letters indicate significant differences within each treatment in different days and large letters indicating significant differences between treatments. (C.P.P.F; Common packaging with low density polyethylene, C.P.NC.F; Common packaging with nano-composite Film)

Discussion

The proximate composition of fish, include lipid, protein, moisture and ash. This composition of the rainbow trout reported in different studies (USDA, 1987; González-Fandos *et al*., 2005) showed some degree of differences, especially for the lipid content. Such variations in the chemical composition of fish is greatly related to the nutrition, catching season (spawning cycles), sexual variation, fish size, living area, as well as the other environmental conditions (Pacheco-Aguilar *et al*., 2000). The compositional variation, due to the reasons mentioned above, may possibly lead to changes in the sensory attributes, including taste, odor, texture, color and surface appearance, which control the acceptability of fish as food (Flick and Martin, 1992; González-Fandos *et al*., 2005). Also, the above mentioned composition may affect the microbial growth (González-Fandos *et al*., 2005). The pH value was reduced in both control and experimental treatments within 10 days, and then, was increased progressively to the end of storage period. The initial decrease in pH may be due to lack of solubility of CO_2 in samples of fish. Increase in CO_2 resulted in decrease in pH (Fan *et al*., 2008). Such results have been observed in other studies (Tiffney and Mills, 1982; Manju *et al*., 2007). Increase in pH within 10 days and then to the end of storage may be due to volatile nitrogen compounds such as ammonia, ammonium, trimethylamine (TMA), produced by internal enzymes activity or microbial enzymes (Riebroy *et al*., 2007). Results showed no significant difference (p>0.05) between control and experimental treatments. Lipid deterioration is often the main cause of a shortened shelf life of fish and fish products, resulting from oxidation (PV and TBA) and enzymatic hydrolysis (FFA) of fatty acids (Pirini *et al*., 2000; Hosseini *et al*., 2010).

Lipid oxidation in fish depends on numerous factors, including type of species, storage temperature, and fat composition (Hernández *et al*., 2009). Primary lipid oxidation was evaluated by means of PV. In this study, PV had increased in both control and experimental treatments during storage. Increase in peroxide value was in control treatment more than experimental treatment, caused by integrating distribution or exfoliation of clay nanoparticles in nano-composite clay/low density polyethylene film. That improves barrier properties relative to O_2, CO_2 and N_2 in experimental group. This result was consistent with findings of Chytiri *et al*. (2004) and Khanipour and Mirzakhani (2012). Thiobarbituric acid (TBA) compounds are the secondary product of lipid oxidation process. TBA values may not give actual rates of lipid oxidation, since malondialdehyde can interact with other components of fish, such as nucleosides, nucleic acid, proteins, amino acids of phospholipids and other aldehydes which are end-products of lipid oxidation (Aubourg, 1993). The rate of increase was more in control group, indicating significant effect of clay nanoparticles in improving the barrier properties of nano-composite film clay/polyethylene in the oxidation process of rainbow trout fillet during storage at refrigerator. There are significant differences (p<0.05) between the early and final days of each treatment, but no significant difference (p>0.05) between the two treatments within 15 days. While, on days 20 and 25, significant differences (p<0.05) were observed between treatments. These results were similar to Chytiri (2004), Rezaei *et al.* (2008), Khanipour and Mirzakhani (2012) and Oguzhan (2013). Fig. 1d shows the amount of volatile nitrogen bases (TVB-N), increased in two treatments of rainbow trout fillet during storage in the refrigerator (4±2) °C, representing the beginning of microbial spoilage reactions. The results of the tests showed that there were significant differences (p<0.05) in each treatment during storage in the refrigerator. Also, any significant differences (p>0.05) was not between the two treatments. TVB-N content of a wide range of basic compounds volatile include ammonia, methylamine, dimethylamine, trimethylamine and other similar compounds, produced by the microbial activities (Rodriguez *et al*., 2008). Similar results are observed in studies of Ozogul *et al*. (2005) on European eel (*Anguilla anguilla*). Total viable counts (TVC) of the rainbow trout fillet has been increased in two treatments (control and experimental) during storage in the refrigerator temperature (4±2) °C. Of course, rate of increase in control treatment was more than experimental treatment; thus, shelf life of fillets of rainbow trout became long in experimental treatment. Maximum Recommended Limit (MRL) for TVC in rainbow trout is 7 logs CFU/g (ICMSF, 1986). The results of this research have been similar to studies performed on rainbow trout stored in ice or refrigerator (Chytiri *et al*., 2004; Rezaei *et al.*, 2008; Khanipour and Mirzakhani, 2012; Oguzhan, 2013) as

well as, similar to researches on (*Epinephelus aeneus*) during 22 days in ice by Ozgoul *et al*. (2008). Psychrophilic total count (PTC), aerobic bacteria such as *Pesudomonas* spp are a group of predominant bacterial in rainbow trout flesh that widely contribute to corruption of fish stored under aerobic conditions (Sousa *et al*., 1996). Permissible bacterial limit for psychrophilic bacteria was 7 log CFU g^{-1} (Gimenez *et al*., 2002). The number of these bacteria in control treatment was 1.7, 7.5 and 9 log CFU g^{-1} on days 0, 20 and 25, respectively; while the number of psychrophilic bacteria in experimental treatment, were 1.57, 6.56 and 7.3 log CFU g^{-1}. The difference is significant (p<0.05) between two treatments. These values are in good agreement with those obtained by (Chytiri *et al*., 2004; Duan *et al*., 2010). The number of lactic acid bacteria (LAB), increased in control and experimental treatments during storage at the refrigerator. The speed of increase of LAB was more in control treatment than the experimental treatment. The significant difference (p<0.05) between the two treatments was observed from day 10 of storage. LAB constitutes a substantial part of the natural micro-flora of fish (Gram and Huss, 1996). This result was similar to study of Sallam (2007) on sliced salmon stored in the refrigerator. Enterobacteriaceae (EBC) is part of the microbial flora inside of fresh rainbow trout stored at 4±2 °C in the refrigerator. Enterobacteriaceae may occur due to cross contamination during post-processing, such as the filleting process (Lindberg *et al*., 1998; Moini *et al*., 2009). The difference was significant (p<0.05) in each treatment between the first and last days of sampling. Moreover, the difference was not significant (p>0.05) between the two treatments, except days 15 and 20. The population of this group was lower than that obtained for other bacteria in this study, which is in agreement with results reported for different fresh Mediterranean fish at the end of the product shelf-life (Gennari *et al*., 1999; Koutsoumanis *et al*., 1999; Ordonez *et al*., 2000; Tejada and Huidobro, 2002) as well, agreement with studies carried out over Atlantic salmon (Amanatudou *et al*., 2000), rainbow trout (Chytiri *et al*., 2004), and Pacific salmon (Sallam, 2007). Although, this group can grow at low temperatures, their abundance decreases during ice or refrigerator storage, possibly because their growth rate is lower than that of other Gram-negative Psychrotrophic spoilers. H_2S producing bacteria, especially *S. putrefaciens*, at the beginning of storing in the refrigerator was 1.52 and 1.46 log CFU g^{-1} for control and experimental treatments, respectively and at the end of storing period, the number of H_2S producing bacteria in two treatments was more than 7 log CFU g^{-1}. This was similar to the results of studies performed about the filleted farmed rainbow trout (Chytiri *et al*., 2004) and in agreement with the results obtained from Vacuum-packaged fillets of Sea bream (*Sparus aurata*) using Gama rays and storing in refrigerator (Chouliara *et al*., 2005). Although a variety of biochemical, physical and

microbiological methods are used to determine fish freshness (Gram and Huss, 1996; Gill, 1992), Sensory evaluation is still the most satisfying method for achieving this goal (Reineccius, 1990; Connell, 1975). Sensory methods are quick and simple and immediately predict qualitative data (Connell, 1975). Based on results from Table 3, using QIM method, factors like skin, color, texture and smell were studied in two treatments, including common packing with low density polyethylene (control treatment) and common packing with clay/low density polyethylene nano-composite film (experimental treatment). Based on the results, the sum of factors in control treatment reached to 5.6 after 15 days of storage, and in experimental group reached to 7.8 after 20 days. As described in Table 1, if the total score is greater than 5, the quality is not acceptable. Therefore, the shelf life of rainbow trout fillet in the control treatment is maximum 15 days and in experimental treatment up to 20 days. The difference between the two treatments was not significant (p>0.05), but the difference within each treatment was significant on days kept in the refrigerator (p<0.05). This result has been similar to research concerning shelf-life of Atlantic salmon (*Salmo salar*) stored in ice with the quality index of 0 to 24 (Sveinsdottir *et al*., 2002). This is also similar to results of researches by Song *et al*. (2011), who studied on packing *Megalobrama amblycephela* using density low polyethylene stored in refrigerator with quality index of 0 to 33. Considering common packing of rainbow trout fillet using low density polyethylene as control group and clay/low density-polyethylene nano-composite film as experimental group, it was shown that nano-composite film, due to lower permeability to O_2 and CO_2, led to delay in lipid oxidation of rainbow trout fillet and increased significantly the shelf life of rainbow trout fillet in the refrigerator. Based on the results obtained from the microbial analysis and their positive correlation with sensory evaluation, shelf life of rainbow trout fillet was 13 to 15 and 18 to 20 days in control and experimental treatments, respectively.

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