Effects of *Capparis spinosa* root extract and modified atmosphere packaging on the shelf life of rainbow trout (*Oncorhynchus mykiss*) fillets by measuring of antioxidant and antimicrobial parameters

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Abstract

The effects of Caper (*Capparis spinosa* L.) extracts on microbiological, chemical and sensory changes of rainbow trout (*Oncorhynchus mykiss*) fillets stored at 4 ˚C were evaluated. Trout fillets were dipped in Caper extracts (0.2 and 0.5%), packaged with or without modified atmosphere packaging (MAP; O_2 5%, CO_2 20%, N_2 75%) and stored at 4 ˚C for 28 days. The data showed that lipid oxidation and spoilage of the samples were significantly delayed in the samples treated with Caper extract associated with MAP ($p<0.05$). The acceptability limit (7 log CFU g^{-1}) of the psychrophilic bacterial counts was observed for MAP+Caper (0.5%) treatment in 7 and 14 days of storage time while it was reported for other treatments only in day 7. The use of 0.5 % Caper+MAP had the best antioxidative and antimicrobial effects, as well as sensory scores in the trout fillets during refrigerated storage (28 days) ($p<0.05$). This study determined that the Caper associated with MAP had significant effects on shelf-life extension of fresh rainbow trout at cold storage period and could be a natural preservative for extending shelf life of fish fillets.

Keywords: Caper extract, Rainbow trout, Modified atmosphere packaging, Shelf life

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Introduction

The rainbow trout (*Oncorhynchus mykiss*), a main member of the Salmonidae, is one of the most important cultured fish in the world. Because of proper characteristics (taste, aroma, white flesh) and nutritional value of the rainbow trout, the demand for this fish has been raised in Iran and other country markets over the past decade (Oraei *et al.*, 2011).

The potent action of autolytic enzymes and microbial activity in the fresh fish makes it as one of the most perishable foods. These putrefactive factors result in the rapid spoilage via changes in biological composition of food matrix (Ashie *et al.*, 1996; Gram and Huss, 1996; Borges *et al.*, 2013; Rodriguez *et al.*, 2013). The spoilage microbes such as *Pseudomonas* spp. (as psychotropic microorganisms) and Enterobacteriacea play crucial role in the refrigerated fish deterioration. These microorganisms could produce several metabolites leading quality loss and reduced shelf-life of this food (Gram and Huss, 1996; Gram and Dalgaard, 2002; Rodriguez *et al.*, 2013). In order to inhibit microbial activity and extend the shelf-life of several food matrices preservation technologies, such as modified atmosphere packaging (MAP) and dipping with different extracts, have been considered.

MAP as a method for food preservation, changes the gas proportions in a food environment via partial withdrawing O_2 or replacing the atmosphere inside the package by a mixture of gases like carbon dioxide (CO_2) and nitrogen (N) gas (Cutter, 2002). CO_2 is a main bacteriostatic gas for fish microflora (Alfaro *et al.*, 2013; Turan and Kocatepe, 2013). It has been reported that high levels of CO_2 could inhibit microbial growth of fish products and extend their shelf-life (Arashisar *et al.*, 2004; Ozogul *et al.*, 2004; Provincial *et al.*, 2013). In addition, O_2 gas is replaced by N_2 (an inert gas) in the package to diminish the growth of aerobic microbes and reduce the oxidative rancidity (Sivertsvik *et al.*, 2002). This technique could extend the shelf-life of fish foods in the refrigerator and maintain the product quality via the inhibition of the normal spoilage-related microbiotic activity (Rodrigues *et al.*, 2016).

Caper (*Capparis spinosa* L.) is a usual member of the genus Capparis (Capparidaceae family) (Tlili *et al.*, 2011). There are more than 250 flowering species in this genus, distributing throughout subtropical to tropical zones of the world (Inocencio *et al.*, 2006). The Caper with characteristics of perennial shrub, thorny, 0.3–1 m tall and 6–10 m roots has different names in the world such as Caper (English), Alaf-e-Mar (Persian), Cappero (Italy) and Alcaparro (Spain) (Nabavi *et al.*, 2016). *Capparis spinosa* (*C. spinosa*) has been found in different parts of the world, from Morocco to Crimea, Armenia and Iran (Nabavi *et al.*, 2016). Phytochemical analysis of this plant indicated that different parts of *C. spinosa* are consist of alkaloids, flavonoids, glucosinolates, phenolic acids, terpenoids, and several studies have thus been investigated the health-promoting effects of this plant and its

active components (Bonina *et al.*, 2002; Tesoriere *et al.*, 2007; Nabavi *et al.*, 2016). Up to now, the cumulative evidences showed that *C. spinosa* possesses various pharmacological effects such as antioxidant, antimicrobial, anticancer and hepato-protective effects (Mishra *et al.*, 2007; Aghel *et al.*, 2010; Gull *et al.*, 2015; Nabavi *et al.*, 2016).

Despite the above mentioned facts, the application of *C. spinosa* root extract as natural preservatives has not been evaluated in the fish products. Therefore, one of the main aims of this project is evaluating antioxidant and antimicrobial component using MAP+Caper on the shelf life of rainbow trout fish fillet during a storage trial at 4±1 °C.

Material and methods

Preparation of C. spinosa root extract
C. spinosa root were prepared from Khave village around Varamin, Iran. Then, the skin of roots was separated and dried at room temperature to a constant weight. All samples were then ground into a fine powder with a blender. The ground samples (30 g) were mixed with 250 mL of 70% ethanol by a shaking water bath for 24 h at 25 °C. The extracts were collected from the solid concentrate after filtering through Whatman paper (No. 1). The residue was re-extracted twice and the all extracts were finally pooled. The ethanol was removed under vacuum at 30 °C by a rotary vacuum evaporator (Laborota 4000, Heidolph, Germany). Then, to prepare dried powder, the samples were dried by a hot air oven at

37 °C for four days. Caper powder was used for preparation of 0.2 and 0.5% (w/v) solutions in distilled water. These extracts were stored at −20 ° C until used.

Preparation and treatment of rainbow trout fish fillets
One hundred rainbow trout fish (*O. mykiss*) with an average weight of 550±50 g were purchased from four farms in Tehran province (Damavand, Poulor, Varamin and Tehran cities), Iran. Fish were placed on the crushed ice and transported to Etka Company (for packaging) within 35 min after catching. Fish were eviscerated, beheaded and filleted under sterile conditions. The packages containing 300 g trout fillets were assembled and divided into 6 treatments including: (1) control, fish fillet samples dipped in distilled water; (2) MAP, fish fillet samples dipped in distilled water and then packaged (Box 42, Henkelman Netherland) by modified atmosphere (O_2 5%, CO_2 20%, N_2 75%) ; (3) Caper 0.2% and (4) Caper 0.5 %, fish fillet samples dipped into 0.2 or 0.5 % of *C. spinosa* root extract for 30 min; (5) MAP + Caper 0.2 % and (6) MAP + Caper 0.5 %, fish fillet samples dipped into 0.2 or 0.5 % of *C. spinosa* root extract for 30 min and then packaged by modified atmosphere. All samples were then drained well and were separately packaged in polyethylene bags and stored at 4±1 °C for storage. Chemical, microbiological and sensory characteristics were investigated during storage at 7, 14, 21 and 28 days.

Determination of peroxide value

The peroxide value (PV) was calculated in the homogenized samples according to Egan *et al.* (1981) using a ferric thiocyanate method. Using ferric iron solution, a standard curve was made to determine the peroxide contents in the samples.

Estimation of malondialdehyde (MDA)

The fillet samples were homogenized with KCl (1.5 %) and the supernatant was utilized for MDA determination as the main thiobarbituric acid reactive substance, according to Monteiro *et al.* (2013). The thiobarbituric acid and trichloroacetic acid were added to the fillet samples, then were incubated at $100°$ C for 15 min. The reaction product was extracted by butyl alcohol and estimated at 535 nm.

Determination of pH

Acid values (pH) were determined according to AOAC (2005). Approximately 10 g gr. fillet in 90 mL distilled water was analyzed for each sample.

Determination of free fatty acid (FFA)

According to Khaniky *et al.* (2015), the fish fillets (20 g) were dissolved in chloroform and then mixed with ethanol. The solutions were titrated by 0.1 N NaOH in the presence of phenolphthalein. Three replicates per sample were titrated. FFA values are usually reported as percentage oleic acid:

% oleic acid=(ml of NAOH×NAOH normality×28.2)/weight of sample (g).

Determination of trimethyl-amine nitrogen

Trimethyl-amine nitrogen (TMA-N) was determined according to Raeisi *et al.* (2016). The fish fillets (25 g) were minced and mixed with distilled water. Their pH was adjusted to 5.2 using 2N HCL. The samples were heated at $70°C$ and then cooled to room temperature. Later, the samples were filtered by Whatman filter paper (No.1). In a micro diffusion dish, 0.025N HCl was transferred to the central compartment. The fillet samples and 35% formaldehyde with saturated K_2CO_3 solution were added into the outer ring. The dish was left at room temperature for 24 h. The HCl in the inner compartment was then titrated with 0.025N NaOH using methyl red/methylene blue indicator. The result was dedicated in mg TMA-N/100 g of samples.

Microbiological analysis

Fish fillets (10 g) were homogenized in a stomacher bag containing 0.85% NaCl. Psychrophilic bacterial counts (PTC) were estimated using plate count agar (Merck, Darmstadt, Germany). Plates of psychrotrophic bacteria were incubated at $7°C$ for 10 days. All counts were offered as log colony-forming units (CFU) g^{-1} and were done in triplicate (Pezeshk *et al.*, 2011; Raeisi *et al.*, 2016).

Sensory evaluation

For sensory evaluation, each fillet sample (100 g) was cooked for 20 min at $98°$ C. The salt (1.5%) was added to the fish fillets. Sensory attributes of the

cooked fillets were evaluated by 10 experienced persons (25–35 years old, male). The sensory parameters were color, odor and taste of fish fillet. According to El-Hanafy *et al.* (2011), the scores for sensory evaluation, in decreasing order, were 10–9 for excellent, 8–7 for good, 6–5 for fair and acceptable, 4–3 for poor and 2–1 for very poor.

Statistical analysis

All the experiments were done in triplicate and data were represented as mean value±standard deviation. One way ANOVA was performed to compare the results and determine if there was any significant difference ($p < 0.05$) between treatments. Duncan's multiple range test was conducted between different groups at the same times. The statistical analysis was used by SigmaStat software version 3.11.0 (Jandel Corp., San Rafael, CA, USA).

Results

Peroxide value

The peroxide values are represented in Fig. 1. In day 7 of storage period, PV was lower in all treatments than the control ($p < 0.05$). The fillets in the treatment of MAP+Caper 0.2% had significantly the lowest PV compared to other groups ($p < 0.05$). In day 14 of storage period, PV was lower in all treatments than the control ($p < 0.05$). The fillets in the treatment of MAP + Caper 0.5% had the lowest PV compared to control, MAP and Caper groups ($p < 0.05$). In day 21 of storage period, PV was lower in all treatments than the control and MAP groups

($p < 0.05$). The fillets in the treatments of MAP +Caper 0.5%, MAP+Caper 0.2%, Caper 0.5% and Caper 0.2% showed significantly the lowest PV respectively ($p < 0.05$). In day 28 of storage period, PV was lower in all treatments than the control ($p < 0.05$). The least PV was significantly belonged to MAP + Caper 0.5%. There was no difference in PV of the fillets in the treatments of Caper 0.2%, Caper 0.5%, and MAP + Caper 0.2% ($p > 0.05$).
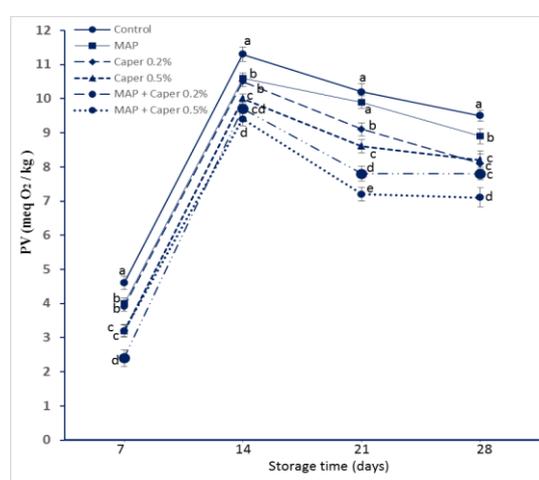

Figure 1: Changes in the peroxide values (PV) in the fish samples during storage time. Control, fish fillet samples dipped in distilled water; MAP, fish fillet samples dipped in distilled water and then packaged by modified atmosphere (O2 5%, CO2 20%, N2 75%); Caper 2% and Caper 5 %, fish fillet samples dipped into 0.2 or 0.5% of C. spinosa root extract; MAP + Caper 0.2% and MAP + Caper 0.5%, fish fillet samples dipped into 0.2 or 0.5% of C. spinosa root extract and packaged by modified atmosphere. Different letters showed significant difference at $p < 0.05$. The letters indicate the variation of treatment for different samples at the same day. Mean value±standard deviation.

TBARS

The data of TBARS formation (MDA) during 28 days of refrigerated storage

time is represented in Fig. 2. The levels of MDA were higher in control than other treatments ($p<0.05$). In days 7, 14 and 21 of storage period, the fillets in the treatments of MAP+ Caper 0.5%, MAP +Caper 0.2%, Caper (0.5 or 0.2%) showed significantly the lowest MDA respectively ($p<0.05$). These statistical differences were also observed between treatments in 28 day except for MAP +Caper 0.5% and MAP +Caper 0.2%.

pH value

Fig. 3 represents changes of pH in control and treatments during storage time. In days 7, 14 and 21 of storage period, The fillets in the groups of MAP +Caper, Caper 0.5%, Caper 0.2%, MAP and control offered significantly the lowest pH respectively ($p<0.05$). In day 28, The least pH was belonged to MAP + Caper treatments and then Caper treatments compared to MAP and control groups ($p<0.05$).
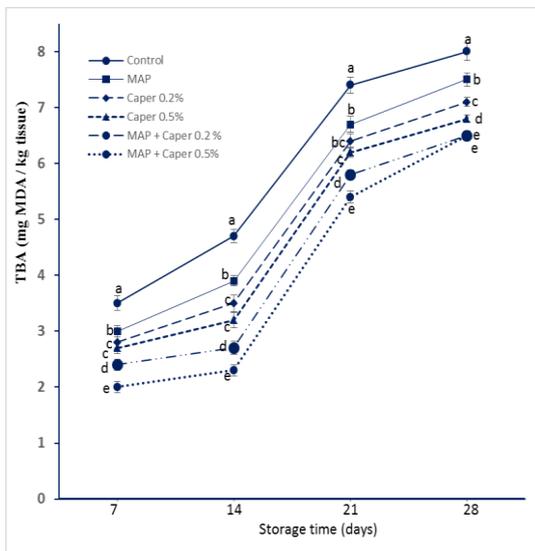

Figure 2: Changes in the TBA reactive substances in the fish samples during storage time. Control, fish fillet samples dipped in distilled water; MAP, fish fillet samples dipped in distilled water and then packaged by modified atmosphere (O_2 5%, CO_2 20%, N_2 75%); Caper 2% and Caper 5 %, fish fillet samples dipped into 0.2 or 0.5% of C. Spinosa root extract; MAP + Caper 0.2% and MAP + Caper 0.5%, fish fillet samples dipped into 0.2 or 0.5% of C. spinosa root extract and packaged by modified atmosphere. Different letters showed significant difference at $p<0.05$. The letters indicate the variation of treatment for different samples at the same day. Mean value±standard deviation.
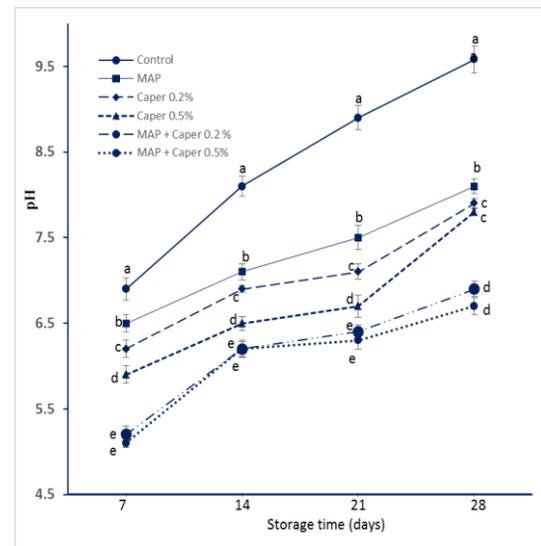

Figure 3: Changes in pH values of rainbow trout fillets during storage time. Control, fish fillet samples dipped in distilled water; MAP, fish fillet samples dipped in distilled water and then packaged by modified atmosphere (O_2 5%, CO_2 20%, N_2 75%) ; Caper 2% and Caper 5 %, fish fillet samples dipped into 0.2 or 0.5% of *C. spinosa* root extract; MAP + Caper 0.2% and MAP + Caper 0.5%, fish fillet samples dipped into 0.2 or 0.5% of C. spinosa root extract and packaged by modified atmosphere. Different letters showed significant difference at $p<0.05$. The letters indicate the variation of treatment for different samples at the same day. Mean value±standard deviation.

Free fatty acids

Fig. 4 represents changes of FFA in the different groups during cold storage time. In day 7, there was no change of FFA levels in the different groups. In days 14, 21 and 28 of storage period, FFA was lower in all treatments than the control ($p<0.05$). In day 21, The fillets in the treatment of MAP + Caper 0.5% showed significantly the lowest PV compared to MAP and Caper groups ($p<0.05$). In day 28, The least FFA was belonged to MAP+Caper treatments and then Caper treatments compared to MAP and control groups ($p<0.05$).

Trimethyl-amine nitrogen

Fig. 5 shows changes of TMA-N values in the different groups during 28 days of storage period. The levels of TMA-N were higher in control than other treatments during storage ($p<0.05$). During cold storage period, the fillets in the treatments of MAP + Caper showed significantly the lowest TMA-N compared to other groups ($p<0.05$). In day 28, the fillets in the treatments of MAP+Caper, Caper 0.5%, Caper 0.2% and MAP showed significantly the least TMA-N values respectively ($p<0.05$).
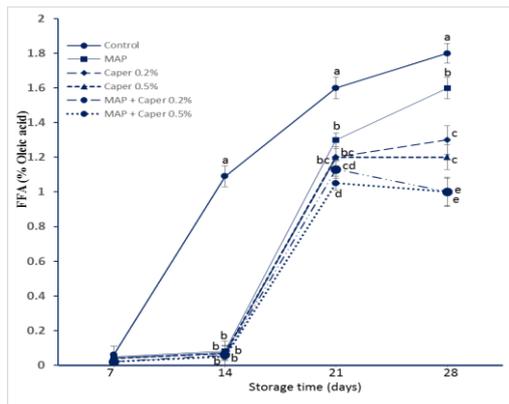

Figure 4: Changes in the total volatile free fatty acid (FFA) in the fish samples during storage time. Control, fish fillet samples dipped in distilled water; MAP, fish fillet samples dipped in distilled water and then packaged by modified atmosphere (O_2 5%, CO_2 20%, N_2 75%); Caper 2% and Caper 5 %, fish fillet samples dipped into 0.2 or 0.5% of C. spinosa root extract; MAP+Caper 0.2% and MAP + Caper 0.5%, fish fillet samples dipped into 0.2 or 0.5% of C. spinosa root extract and packaged by modified atmosphere. Different letters showed significant difference at $p<0.05$. The letters indicate the variation of treatment for different samples at the same day. Mean value±standard deviation.
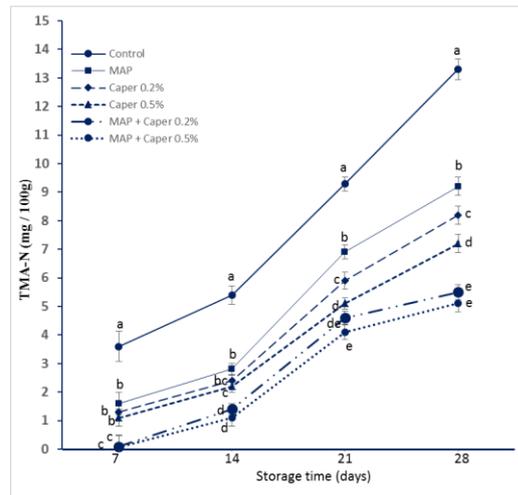

Figure 5: Changes in the trimethyl-amine nitrogen (TMA-N) in the fish samples during storage time. Control, fish fillet samples dipped in distilled water; MAP, fish fillet samples dipped in distilled water and then packaged by modified atmosphere (O_2 5%, CO_2 20%, N_2 75%); Caper 2% and Caper 5 %, fish fillet samples dipped into 0.2 or 0.5% of C. spinosa root extract; MAP+Caper 0.2% and MAP+ Caper 0.5%, fish fillet samples dipped into 0.2 or 0.5% of C. spinosa root extract and packaged by modified atmosphere. Different letters showed significant difference at $p<0.05$. The letters indicate the variation of treatment for different samples at the same day. Mean value±standard deviation.

Psychrophilic bacterial count

Fig. 6 showes changes of PTC in the fish samples during storage time. The PTC was lower in samples of MAP + Caper groups than control and MAP groups after 7 days of cold storage.
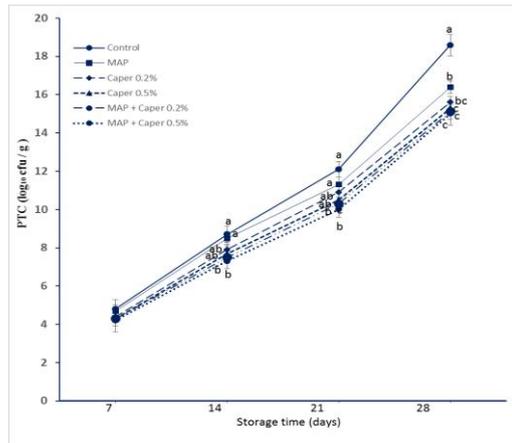

Figure 6: Changes in psychrophilic bacterial counts (PTC) in the fish samples during storage time. Control, fish fillet samples dipped in distilled water; MAP, fish fillet samples dipped in distilled water and then packaged by modified atmosphere (O_2 5%, CO_2 20%, N_2 75%); Caper 2% and Caper 5 %, fish fillet samples dipped into 0.2 or 0.5% of C. Spinosa root extract; MAP+Caper 0.2% and MAP + Caper 0.5%, fish fillet samples dipped into 0.2 or 0.5% of *C. spinosa* root extract and packaged by modified atmosphere. Different letters showed significant difference at *p*<0.05. The letters indicate the variation of treatment for different samples at the same day. Mean value±standard deviation.

Sensory evaluation

Figs. 7, 8 and 9 represent odor, taste and color property of the fish samples during storage time. The fillet samples treated with the Caper extract+MAP had significant better sensory scores than the control or MAP samples (*p*<0.05).
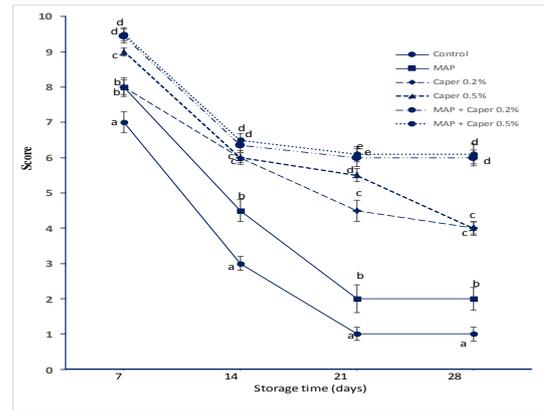

Figure 7: Odor property of the fish samples during storage time. Control, fish fillet samples dipped in distilled water; MAP, fish fillet samples dipped in distilled water and then packaged by modified atmosphere (O2 5%, CO2 20%, N2 75%); Caper 2% and Caper 5 %, fish fillet samples dipped into 0.2 or 0.5% of *C. spinosa* root extract; MAP + Caper 0.2% and MAP + Caper 0.5%, fish fillet samples dipped into 0.2 or 0.5% of *C. spinosa* root extract and packaged by modified atmosphere. Different letters showed significant difference at *p*<0.05. The letters indicate the variation of treatment for different samples at the same day. Mean value±standard deviation.

Figure 8: Taste property of the fish samples during storage time. Control, fish fillet samples dipped in distilled water; MAP, fish fillet samples dipped in distilled water and then packaged by modified atmosphere (O2 5%, CO2 20%, N2 75%); Caper 2% and Caper 5 %, fish fillet samples dipped into 0.2 or 0.5% of *C. spinosa* root extract; MAP + Caper 0.2% and MAP + Caper 0.5%, fish fillet samples dipped into 0.2 or 0.5% of *C. spinosa* root extract and packaged by modified atmosphere. Different letters showed significant difference at *p*<0.05. The letters indicate the variation of treatment for different samples at the same day. Mean value±standard deviation.
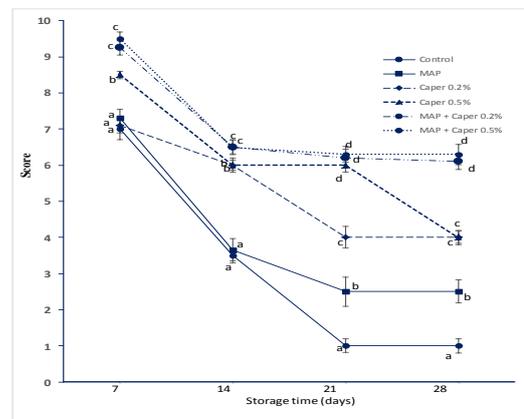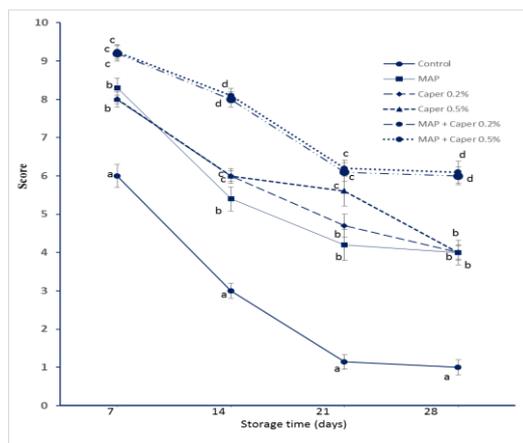
Figure 9: Color property of the fish samples during storage time. Control, fish fillet samples dipped in distilled water; MAP, fish fillet samples dipped in distilled water and then packaged by modified atmosphere (O_2 5%, CO_2 20%, N_2 75%); Caper 2% and Caper 5 %, fish fillet samples dipped into 0.2 or 0.5% of *C. spinosa* root extract; MAP+ Caper 0.2% and MAP+ Caper 0.5%, fish fillet samples dipped into 0.2 or 0.5% of *C. spinosa* root extract and packaged by modified atmosphere. Different letters showed significant difference at *p<0.05*. The letters indicate the variation of treatment for different samples at the same day. Mean value±standard deviation.

Discussion

The fillets of rainbow trout fish is very sensitive to lipid oxidation during refrigerated storage because of high levels of the polyunsaturated (26 %) and monounsaturated fatty acids (50 %) (Kotakowska *et al.*, 2006), leading to the decline of quality and undesirable odor formation (Venugopal and Shahidi, 1996). The data confirmed that the lowest peroxide values were in the groups of MAP + Caper 0.2 or 0.5% which could be due to the antioxidant property of the plant materials. The antioxidant property of Caper extracts is related to flavonoids, terpenoids and tannins (Nabavi *et al.*, 2016). It has

been considered that the maximum acceptable limit of peroxide value for foodstuffs is 10–20 mEq O_2 kg^{-1} oil according to Kotakowska *et al.* 2006. In our study, the peroxide value in the groups of MAP + Caper 0.2% and MAP + Caper 0.5%, was 9.7 and 9.4 mEq O2/kg respectively. Samples with MAP + Caper 0.5% obviously showed the best performance in primary lipid oxidation retardation after 28 days. In agreement with these data, Pezeshk *et al.* (2011) reported that shallot and turmeric extracts were efficient in retarding the production of primary lipid oxidation products in rainbow trout fillets stored at 4±1 ˚C. Raeisi *et al.* (2016) also indicated that the lipid oxidation was significantly delayed in samples treated with a cumin (6.0 %) extract.

The malondialdehyde as an index of lipid oxidation was evaluated. This index is one of the main end-products of lipid oxidation (Weber *et al.*, 2008). According to the previous studies, MDA values of 1–2 mg kg^{-1} of fish fillet are usually regarded as an acceptable limit (Connell, 1990). In the present study, MDA for the control and all treatments were higher than such proposed limits after 7 days of storage. However, the antioxidant ability of Caper compounds reduces free radicals and resulting in the significant reduction of MDA in the treated groups especially MAP + Caper 0.2% and MAP + Caper 0.5% groups at the all times of storage (Ojagh *et al.*, 2010).

In the current study, it has been indicated that MAP + Caper 0.2% and MAP + Caper 0.5% treatments via

maintaining acid values at low levels, had the highest significant effects against lipid oxidation during cold storage times. When fats undergo putrefaction, triglycerides are changed into fatty acids and glycerol which therefore elevate the pH value of samples. These preservative effects of Caper is likely due to its phenolic compounds, which play a crucial role in stabilization and reduction of lipid oxidation (Mkaddem *et al.*, 2009; Chen *et al.*, 2014).

FFA measurement is one of the methods to determine the degree of fish decomposition. Formation of FFA, a result of enzymatic and non-enzymatic lipid hydrolysis is applied as an indicator of the lipid quality. FFA formation is result of fat catalysis by endogenous enzymes. According to our data, treatments with Caper were more efficient in delaying the production of FFA especially after 21 days of storage period. Khaniky *et al.* (2015) also reported a reduction of FFA in trout fillets during refrigerated storage after dipping in the pomegranate seeds extract. This extract also has an antioxidant effects.

In the stored fish fillets, enzymatic activities and bacterial spoilage reduce the trimethylamine oxide (TMAO) to Trimethylamine (TMA) (Frangos *et al.*, 2010). Frangos *et al*. (2010) reported that if the initial TMA-N values (day 0) of trout fillets were about 1.48– 1.63 mgN $100g^{-1}$ the fleshes have good quality. In the present study, TMA-N value was lower in all MAP and Caper treatments at day 7 of storage, and was lower than 1.63 mgN $100g^{-1}$ indicating

good shelf life of rainbow trout fillets in this time. There are different reports of TMA acceptability limits which were influenced by fish species, seasons, initial bacterial count and storage conditions (Connell, 1990). The TMA acceptability limits for many fish species are including: 5 mgN $100g^{-1}$ for sea bass; 5–10 mgN $100g^{-1}$ for sardines; 12 mgN $100g^{-1}$ for hake (Raeisi *et al.*, 2016). In the present study, the minimum TMA (0.07-5.5 mgN $100g^{-1}$) was belonged to MAP + Caper 0.2% and MAP + Caper 0.5%. During cold storage period of fish fillets, the gram-negative psychrotrophic bacteria are the important microorganisms which cause aerobically spoilage (El-Hanafy *et al.*, 2011). Our data indicated that Caper extract in combination with MAP reduces psychrotrophic bacteria. This result could be due to antibacterial activity of Caper extract. Raeisi *et al.* (2016) also showed that wild mint leaf extract could reduce PTC of trout fish fillets during cold storage.

Sensory evaluation is a test that is dependent on the senses to estimate the food quality. Human consumption of fish products is mostly depended to the sensory attributes. According to this test, the quality of fish fillets would be acceptable for human consumption when the sensory score is between 4 and 10 (El-Hanafy *et al.*, 2011). During storage time, quality of fish fillets decreased in all samples. The fillet samples treated with the Caper extract + MAP had better sensory scores. Treated samples with a higher level of plant extract (0.5%) exhibited no better

sensory qualities than those treated with 0.3% plant extract.

However, the results of sensory evaluation and microbial / chemical quality analyses supported each other in the present study. Previous studies have also shown that herbal extracts could improve both sensory attributes of fillets and microbial / chemical qualities via their antioxidant and antimicrobial activity and increase the fish shelf life (Frangos *et al.*, 2010; Ojagh *et al.*, 2010; Raeisi *et al.*, 2016).

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