Effect of *Salvia officinalis* L. extract on chemical, microbial, sensory and shelf life of rainbow trout fillet

Tooraj Mehdizadeh¹ · Hossein Tajik¹ · Sima Jafarie¹ · Ata Kaboudari¹

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Abstract The present study aimed at extending shelf life of rainbow trout fillet. Fish and seafoods are the most valuable nutrients, however, high levels of moisture, free amino acids, unsaturated fatty acids, and the content of nutrients make them to be categorized as spoilable and unstable foods. The objective of this research was to study of the shelf life of rainbow trout fillets using *Salvia officinalis* L. extract. Chemical and microbial analyses were performed on treatments. The usage of hydro-ethanol extract of *S. officinalis* L. significantly decreased microbial growth in fish fillet and there were significant differences among concentrations of the extract ($p \leq 0.05$). The presence of extract of *S. officinalis* L. resulted in an inhibitory effect on the formation of TVB-N and FFA in comparison with the control group at all analytical times. In conclusion, the shelf life of fish fillets was increased noticeably using extract at 4 ± 1 °C.

Keywords *Salvia officinalis* L. · Extract · Rainbow trout fillet · Shelf life

Introduction

Fish and seafood are the most valuable nutrients, however, high levels of moisture, free amino acids, unsaturated fatty acids, and the content of nutrients make them to be categorized as spoilable and unstable foods (Cakli et al., 2007). In fact, fish meat putrefaction is the result of biological reactions such as lipid oxidation, effect of the enzymes and the metabolic activity of microorganisms (Khalafalla et al., 2015). Regarding the increasing awareness, consumers prefer to consume food containing natural preservatives instead of chemical preservatives, and the natural preservatives are nowadays more popular (Holley and Patel, 2005). On the other hand, consumers are looking for processes and methods keep maintaining nutrients in food (Nagarajarao, 2016).

Rainbow trout (*Oncorhynchus mykiss*) is the most important cultured species of freshwater salmon (*Salmonidae*). It naturally has a high content of unsaturated fatty acids, such as Eicosapentaenoic Acid (EPA) and Docosahexaenoic Acid (DHA), that are very useful for human health. The importance of rainbow trout is because it is rich in unsaturated long chain fatty acids that could be useful in controlling cardiovascular diseases, cancers, Alzheimer's, infectious diseases and blood pressure (Aubourg, 2001; Barat et al., 2006). It is not always possible to supply fresh fish in the market, therefore, during storage, lipid oxidation produces unpleasant smells, undesirable tastes and reduces the nutritional value of the fish (Begin and Calsteren, 1999).

About a one-third of the world food production is lost every year due to microbial and chemical spoilage (Lund et al., 2000). Various methods have been using to store foods, such as heating, cooling, drying, salting and other methods. Recently, the use of active packaging containing

✉ Tooraj Mehdizadeh
T.Mehdizadeh@Urmia.ac.ir;
Tooraj.mehdizadeh@yahoo.com

Hossein Tajik
H.tajik@Urmia.ac.ir

Sima Jafarie
sima_514@yahoo.com

Ata Kaboudari
A.kaboudari@urmia.ac.ir

¹ Department of Food Hygiene and Quality Control, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran

active antimicrobial and antioxidant compounds such as essential oils and herb's extracts in foods, are being explored and developed (Mehdizadeh et al., 2012).

Salvia officinalis L. is one of the most valuable plants that is widely used in traditional medicine (Bensid et al., 2014). The essential oil and extracts of this plant bear antimicrobial, antiviral, cytotoxic, anti-mutagenic, anti-inflammatory and anti-fungal properties. Some phenolic compounds of plants belonging to this genus bear antimicrobial activity and excellent antioxidant capacity (Bisio et al., 1997). The extract of the herb contains a wide range of phenolic compounds, flavonoids and phenolics that may contribute to total antioxidant activity (Bremner, 2002). According to the studies, it is possible that these compounds in this plant are useful in fish preservation. The aim of the present study was to effect of the *S. officinalis* L. extract to extend the shelf life of rainbow trout fillets.

Materials and methods

Preparation of fish samples

Fishes with an average weight of 1100 ± 40 g were transferred to the laboratory alongside the ice. Then, the viscera were immediately evacuated. Then, they were washed with drinking water to remove blood and viscous layers on the fillets.

Preparation of ethanolic extract of *Salvia officinalis* L. and determination of its constituents

Cold Aqueous Percolation was used to prepare the extract. For this purpose, the plant was prepared from the Agricultural and Natural Resources Research Center, Ardabil Province—Iran. Then was cleaned, washed and air dried in a dark place. It was turned to powder form using a mill. Thousand gram of powder was dissolved in 5 L ethanol alcohol (30 °C) and placed in a Percolator for 72 h. Then the solvent was added to the plant mass and uniformly penetrated into the entire plant mass and continued for 48 to 72 h of percolation action. So that the solvent completely covered the plant mass. For filtration, Whatman No. 1 filter paper was used and extract solution was dried under reduced pressure. Finally, the extract was stored in the refrigerator until use (Kanerian et al., 2012).

Preparation of treatments

Filletts prepared for coating were randomly selected and prepared in 4 treatments (control, 2%, 4% and 6% extracts) and after 10 s immersion were placed in polyethylene bags

and kept for 25 days at refrigerated (temperature 4 ± 1 °C) for microbial, chemical and sensory tests.

Proximate composition of fillets

To determine the approximate composition, the whole fillet was turned by the machine and was completely homogeneous. To determine the amount of ash, electric furnace at 550–500 °C and the drying method in the oven was used (Chang et al., 1977). Total Protein was measured by Kjeldahl method and the amount of nitrogen was multiplied by 6.25 and was considered as protein percentage. The amount of fat was measured using Soxhlet method (Chen et al., 2008), using ether petroleum solvent and Soxtec device (ST 255 Soxtec, Denmark). These experiments were performed for determining the chemical composition of fish fillets.

Measurement of total volatile basic nitrogen (TVB-N)

The Goulas and Kontominas (2007) methods were used to measure the total volatile basic nitrogen. Ten gram of a fillet sample was blended with 2 g magnesium oxide and 300 mL of distilled water and heated in the Kjeldahl balloon. Distilled vapours were collected in an erlenmeyer flask containing 25 mL Boric acid with a purity of 3% and a few drops of a color-coded mixture containing 0.1 g of Methyl Red and 0.1 g of Methylene Blue in 100 mL of ethanol. Then, it was titrated with sulphuric acid 0.05 M. The amount of volatile volumes was calculated based on the amount of sulfuric acid used and expressed as mg of nitrogen per 100 g of fish fillet.

Measurement of thiobarbituric acid (TBA)

Thiobarbituric acid test was performed based on a method described by Etemadian et al. (2011). In brief, 10 g of fillet were homogenized with 97.55 mL distilled water and 2.5 mL hydrochloric acid, 4 N. Then, 3 drops of silicon and several glass pearls were added. 5 mL of vapours with 5 mL of thiobarbituric acid were mixed and boiled for 1 min. Then, the absorbance was measured at 538 nm with a spectrophotometer. The amount of thiobarbituric acid was expressed in mg malondialdehyde (MDA) per kg of sample.

Measurement of free fatty acids (FFA)

Measurement of free fatty acids was based on a method described by Egan et al. (1997). In brief, 0.2 g of fat extracted from fish fillet was dissolved in 50 mL of solvent, a mixture of equal volume of ethanol 96% and diethyl

ether. Then, 1 to 2 drops of Phenolphthalein were added as a colour detector. The sample was titrated with sodium hydroxide (0.1 N) by a digital burette. The amount of free fatty acid was calculated according to the following equation:

$$\text{FFA} = \frac{V \times 28/2 \times 100}{W \times 1000}$$

V, volume of sodium hydroxide; W, sample fat content.

1. Measurement of pH

Five gram of homogenized fillet were mixed with 10 mL of distilled water and finally the pH of the sample was measured using a pH-meter (Chytiri et al., 2004).

Microbiological analysis

For measuring of total bacterial (Plate Count Agar media) and Pseudomonas (Cetrimide Agar media), plates were kept at 37 °C for 24 and 48 h, respectively, in the incubator and then the number of colonies in the plates were counted. For counting of Psychrophilic bacteria, they were cultured in Plate count agar media and incubated 5 days at 15 °C. For detection of Enterobacteriaceae, Violet red bile agar media plates were incubated for 24-h at 37 °C, and then large colonies with violet clouds were counted. To measure the amount of H₂S producing bacteria, the samples were cultured in Iron agar media and the plates were incubated at 30 °C for 3 days, then black colonies were counted (Cava et al., 2006).

Sensory evaluation

For sensory evaluation of the fillets, a 7-person trained panel was used. Sensory evaluation was done on colour, odor, texture and total acceptability. The fresh fillet was stored at − 20 °C and was considered as the highest score at the time of sensory evaluation. Sensory evaluation was performed under similar optical and temperature conditions. For scoring, a scale of 0 to 9 was used that 9 had the highest score and 0 had the lowest score. A product with a score of < 6 was defined as an unacceptable product (Coma et al., 2001).

Statistical analysis

Data were analyzed using SPSS software (IBM SPSS Statistics for Windows, version 19.0). First, the data were normalized using the Kolmogorov–Smirnov test and then the data were analysed using ANOVA and Duncan test. Significant differences were observed between treatments at 5% probability level.

Results and discussion

Proximate composition of fillets

The values for rainbow trout fillet analysis included moisture 72.8%, protein 19.5%, fat 3.4% and ash 1.7%. These values were measured for 3 fillet samples selected randomly among the samples at the beginning of the period. In this study, the chemical analysis of fillet compounds was initially carried out. There are several studies in the literature and almost all of these studies have reported different amounts of fish body compositions, especially fat contents. Differences in chemical composition of fish fillets can be related to feeding, season of fishing, sex differences, fish size and living environment. Differences in these compounds, in particular the amount of fat, can lead to changes in sensory characteristics such as taste, smell, texture, colour, and apparent characteristics that determine the acceptability of fish (González-Fandos et al., 2005; Ibrahim Sallam, 2007).

Microbiological analyses

The result of the total count of mesophilic bacteria, Psychrophilic bacteria, pseudomonas bacteria, H₂S producing bacteria and total number of enterobacteriaceae bacteria are shown in Table 1. According to the results of microbial analyses, use of hydro-ethanol extract of *S. officinalis* L. has significantly decreased microbial growth in salmon fillet covered with this material during storage in the refrigerator, which was higher at higher levels of the extract. In the case of total bacterial count, the difference in microbial growth among the four groups was analyzed so that from day 5 the difference between treatments with the levels of extract with the control group was increased on days 10, 15 and 20. The difference between all groups was significant and in general, the 6% group showed a lower microbial growth than the other three groups ($p < 0.05$). Significant differences ($p < 0.05$) were observed in the count of live mesophilic bacteria between the treated groups with different levels of extract and control group. As mentioned, at 6% level of the extract, the number of bacteria was lower than the other groups. In the case of Psychrophilic bacteria, the difference in their count in treated groups with hydro-ethanol extract of *S. officinalis* L. was significant ($p < 0.05$). On days 5 and 10 the difference was significant among all groups, however, on days 15, 20 and 25 the difference was not significant among groups with 4 and 6% of extract ($p > 0.05$). Similar results were observed for Pseudomonas bacteria, so that, increases in the concentration of the extract decreased the number of bacteria, although the difference between the control group

Table 1 Microbial changes of fish fillet during storage period

Treatments	Storage time (days)					
	0	5	10	15	20	25
<i>Mesophilic bacteria</i>						
Control	5.11 ± 0.15 ^{a*}	6.47 ± 0.26 ^a	7.20 ± 0.15 ^a	7.82 ± 0.12 ^a	9.50 ± 0.40 ^a	9.50 ± 0.40 ^a
2% SE	5.29 ± 0.10 ^a	6.69 ± 0.30 ^a	7.04 ± 0.16 ^a	7.46 ± 0.15 ^c	8.60 ± 0.10 ^b	8.60 ± 0.10 ^b
4% SE	4.75 ± 0.10 ^b	5.30 ± 0.21 ^b	5.47 ± 0.22 ^b	5.39 ± 0.11 ^b	6.01 ± 0.15 ^c	6.01 ± 0.15 ^c
6% SE	2.04 ± 0.22 ^d	2.30 ± 0.15 ^d	2.74 ± 0.13 ^d	3.52 ± 0.21 ^c	3.67 ± 0.05 ^c	3.67 ± 0.05 ^c
<i>Pseudomonas spp.</i>						
Control	4.01 ± 0.11 ^a	5.18 ± 0.14 ^a	5.47 ± 0.13 ^a	6.01 ± 0.16 ^a	5.91 ± 0.25 ^a	5.91 ± 0.25 ^a
2% SE	3.21 ± 0.20 ^c	3.53 ± 0.11 ^b	3.98 ± 0.09 ^c	5.55 ± 0.05 ^c	5.48 ± 0.18 ^a	5.48 ± 0.18 ^a
4% SE	2.53 ± 0.12 ^b	3.14 ± 0.40 ^b	3.56 ± 0.22 ^b	3.94 ± 0.13 ^b	3.44 ± 0.12 ^b	3.44 ± 0.12 ^b
6% SE	1.42 ± 0.21 ^d	1.59 ± 0.21 ^c	2.61 ± 0.24 ^d	2.71 ± 0.14 ^c	1.74 ± 0.11 ^d	1.74 ± 0.11 ^d
<i>Enterobacteriaceae</i>						
Control	3.19 ± 0.11 ^a	5.30 ± 0.09 ^a	6.83 ± 0.02 ^a	7.58 ± 0.07 ^a	7.47 ± 0.05 ^a	7.47 ± 0.05 ^a
2% SE	3.09 ± 0.21 ^a	4.24 ± 0.06 ^c	6.43 ± 0.16 ^c	7.11 ± 0.25 ^c	3.09 ± 0.12 ^c	3.09 ± 0.12 ^c
4% SE	2.05 ± 0.25 ^b	2.30 ± 0.14 ^b	2.17 ± 0.21 ^b	3.57 ± 0.24 ^b	2.68 ± 0.13 ^b	2.68 ± 0.13 ^b
6% SE	1.31 ± 0.14 ^c	1.92 ± 0.17 ^d	1.40 ± 0.06 ^d	2.26 ± 0.13 ^c	1.29 ± 0.15 ^c	1.29 ± 0.15 ^c
<i>Psychrotrophic bacteria</i>						
Control	5.17 ± 0.18 ^a	7.06 ± 0.06 ^a	8.19 ± 0.12 ^a	8.72 ± 0.17 ^a	8.65 ± 0.26 ^a	8.65 ± 0.26 ^a
2% SE	3.90 ± 0.30 ^b	5.30 ± 0.31 ^c	7.38 ± 0.06 ^d	8.05 ± 0.15 ^c	8.49 ± 0.19 ^a	8.49 ± 0.19 ^a
4% SE	2.73 ± 0.17 ^c	3.84 ± 0.24 ^b	4.66 ± 0.23 ^c	5.29 ± 0.23 ^b	8.79 ± 0.14 ^a	8.79 ± 0.14 ^a
6% SE	3.05 ± 0.16 ^d	3.82 ± 0.14 ^b	4.04 ± 0.18 ^c	3.09 ± 0.19 ^c	4.78 ± 0.11 ^c	4.78 ± 0.11 ^c
<i>H₂S producing bacteria</i>						
Control	5.17 ± 0.18 ^a	7.06 ± 0.06 ^a	8.19 ± 0.12 ^a	8.72 ± 0.17 ^a	8.65 ± 0.26 ^a	8.65 ± 0.26 ^a
2% SE	3.90 ± 0.30 ^b	5.30 ± 0.31 ^c	7.38 ± 0.06 ^d	8.05 ± 0.15 ^c	8.49 ± 0.19 ^a	8.49 ± 0.19 ^a
4% SE	2.73 ± 0.17 ^c	3.84 ± 0.24 ^b	4.66 ± 0.23 ^c	5.29 ± 0.23 ^b	8.79 ± 0.14 ^a	8.79 ± 0.14 ^a
6% SE	3.05 ± 0.16 ^d	3.82 ± 0.14 ^b	4.04 ± 0.18 ^c	3.09 ± 0.19 ^c	4.78 ± 0.11 ^c	4.78 ± 0.11 ^c

*Different lower case letters in a column indicate significant differences between treatments ($p < 0.05$)

and the 2% treatment until the end of day 15 was not statistically significant ($p > 0.05$). The difference in the number of H₂S-producing bacteria was statistically significant among all the analyzed groups from day 10 onwards ($p < 0.05$). The results of the measurement of enterobacteriaceae bacteria showed that the bacterial population in both control and extract samples was generally low, however, their populations in the control samples were more than those containing the extract. Although enterobacteriaceae can grow at a lower temperature, they are not much increased during storage compared to psychrophilic bacteria. Such a condition could be probably related to their slower growth compared to other gram-negative psychrophilic bacteria that cause spoilage and reduce oxygen. Similar results are reported by other researchers (Chytiri et al., 2004; Papadopoulos et al., 2003).

Chemical evaluations

The results of total volatile basic nitrogen (TVB-N) test and thiobarbituric acid (TBA) test are shown in Table 2. The TVB-N depends on the bacterial growth and the activity of the internal enzymes. This value is one of the criteria for the decomposition and destruction of meat and its proteins, which is more than ammonia and volatile amines (Özden et al., 2007). At the start of the experiment, the TVB-N was 11.54–11.84 mg per 100 g of fish fillet. This was consistent with the amount reported by others who reported that TVB-N in fresh fish fillet between 5 and 20 mg of nitrogen per 100 g (Connell, 1990). In the present study, TVB-N was increased in the course of time. The presence of hydro-ethanol extract of *S. officinalis* L. caused an inhibitory effect on the formation of TVB-N in comparison with the control group at all analytical times. Some researchers reported that an acceptable amount of TVB-N for salmon fillet is 25 mg TVB-N per 100 g (Arashisar et al., 2004). The difference in the amount of TVB-N between the coated specimens with the level of 6% of the

Table 2 Total volatile basic nitrogen (TVB-N) and thiobarbituric acid (TBA) in different treatments during storage in refrigerator ($4 \pm 1^\circ\text{C}$)

Treatments	Storage days					
	0	5	10	15	20	25
<i>TVB-n</i>						
Control	$11.72 \pm 0.03^{\text{a}}$	$16.62 \pm 0.01^{\text{a}}$	$18.38 \pm 0.01^{\text{a}}$	$26.89 \pm 0.12^{\text{a}}$	$32.11 \pm 0.11^{\text{a}}$	$45.23 \pm 0.4^{\text{a}}$
2% SE	$11.84 \pm 0.57^{\text{a}}$	$15.25 \pm 0.22^{\text{b}}$	$16.66 \pm 0.36^{\text{b}}$	$25.11 \pm 0.06^{\text{b}}$	$29.23 \pm 0.29^{\text{b}}$	$45.11 \pm 0.12^{\text{b}}$
4% SE	$11.57 \pm 0.05^{\text{a}}$	$12.87 \pm 0.00^{\text{c}}$	$13.63 \pm 0.11^{\text{c}}$	$21.18 \pm 0.25^{\text{c}}$	$24.15 \pm 0.00^{\text{c}}$	$33.28 \pm 0.35^{\text{c}}$
6% SE	$11.54 \pm 0.29^{\text{a}}$	$12.59 \pm 0.53^{\text{c}}$	$13.84 \pm 0.27^{\text{d}}$	$18.71 \pm 0.20^{\text{d}}$	$21.78 \pm 0.01^{\text{d}}$	$26.16 \pm 0.15^{\text{d}}$
<i>TBA</i>						
Control	$0.10 \pm 0.02^{\text{a}}$	$0.47 \pm 0.25^{\text{a}}$	$0.63 \pm .17^{\text{a}}$	$1.15 \pm 0.02^{\text{a}}$	$1.10 \pm 0.23^{\text{a}}$	$1.21 \pm 0.05^{\text{a}}$
2% SE	$0.11 \pm 0.05^{\text{a}}$	$0.38 \pm 0.50^{\text{b}}$	$0.45 \pm 0.11^{\text{b}}$	$0.87 \pm 0.15^{\text{b}}$	$0.93 \pm 0.13^{\text{b}}$	$1.02 \pm 0.41^{\text{b}}$
4% SE	$0.07 \pm 0.01^{\text{a}}$	$0.19 \pm 0.11^{\text{c}}$	$0.25 \pm 0.15^{\text{c}}$	$0.34 \pm 0.01^{\text{c}}$	$0.55 \pm 0.03^{\text{c}}$	$0.56 \pm 0.01^{\text{c}}$
6% SE	$0.08 \pm 0.37^{\text{a}}$	$0.14 \pm 0.03^{\text{d}}$	$0.22 \pm 0.28^{\text{c}}$	$0.37 \pm 0.10^{\text{c}}$	$0.42 \pm 0.36^{\text{d}}$	$0.49 \pm 0.12^{\text{d}}$

*Different lower case letters in a column indicate significant differences between treatments ($p < 0.05$)

extract with the control group was significant at all experiments times ($p < 0.05$). Reduction of TVB-N formation in extracts treated samples was relevant with antimicrobial activity of extracts (Burt, 2004; Zolfaghari et al., 2010).

The results of the present study showed that during the storage period of rainbow trout, the TBA index was increased to about 1.5 mg malondialdehyde per kg of sample. The limiting value of this index is not stated with certainty, due to the lack of its increasing trend until the end of the storage time, however, the amount of 1–2 mg is the limiting value of this index (Goulas and Kontominas, 2007). The lowest amount of TBA, 0.07 mg MDA/kg, in the present study was at the beginning of the experiment for fish samples. TBA values were increased in all experimental groups and there was no significant difference between groups of 4 and 6% until day 15 ($p > 0.05$). However, on days 20 and 25, there was a significant difference among all groups ($p < 0.05$). By day 15, the trend for the index was high and from the day 15 onward, it was lower (Silva and Ammerman, 1993). The ethanolic extract of *S. officinalis* L. has been reported to contain 6% carnosic acid and 6.9% rosmarinic acid and flavonoids such as Routin and Apigenin with antagonist effects (Durling et al., 2007).

The results of free fatty acids (FFA) test and pH measurements are shown in Figs. 1 and 2, respectively. The results of the FFA in samples during storage showed that the amount of volatile fatty acids were increased over the time, that could be due to the enzymatic hydrolysis of fish fats during storage at refrigerator temperatures (Serdarglu and Felekoglu, 2005). The findings of the present study showed that the treatments containing extract produced less

Fig. 1 Results of free fatty acids (FFA)

Fig. 2 Results of pH measurement

FFA than the control group, which was significant at 6% in most days ($p < 0.05$).

The pH of all samples was increased during storage and the highest increase was observed in control sample. Furthermore, the difference between the control and the

treatments was significant ($p \leq 0.05$). In addition to the production of nitrogen compounds, such as ammonia, trimethylamine and initial oxidation products, the pH of fish muscle was increased during storage. Accordingly, it could be considered as an indicator of the spoilage (Thaker et al., 2017). Chamanara et al. (2012) reported that the increase in trend of pH was inhibited in trout coated with chitosan-containing thymus essential oil. In addition, the use of Clove essential oil was able to keep the Cod fish fillet pH below 7 (Gómez-Estaca et al., 2007).

Sensory evaluation

In the present study, according to the result of sensory evaluation (Table 3), there was a decrease in odor acceptability in all groups with different levels of the extract, especially at high levels on early days of storage in the refrigerator ($p > 0.05$). In the whole experiments, the control group received a lower rating in terms of all attributes. Meanwhile, the extracts, especially the higher levels, showed higher acceptability ($p < 0.05$). In most cases, the maintenance period for these groups was still within a range between 15 and 20 days in terms of sensory characteristics.

In various studies, different methods for storing of marine foods have been investigated. Temperature and freezing can affect the level of microbial and chemical deterioration (Jessen et al., 2014). In recent years due to advances in food storage, the use of essential oils and extracts has become very important (Hassoun and Emir Çoban, 2017). In a study, some extracts and essential oils, cinnamon, thyme and rosemary, with anti-microbial and antioxidant effects were introduced and reported to be very useful in the storage of seafood, such as fish, (Yu et al., 2016). Altinelataman et al. (2015) have reported results that sage extract and essential oil has the ability to extend the shelf life of Sea bass (*Dicentrarchus labrax* L.) fillet higher than 6 days in comparison with the control group. This finding is slightly lower compared to the current study. The reason for this difference is in the family, fat content, and the way in which the plant extract is used in the coating solution. In a study by Yu et al. (2016) it was found that grass carp fillet covered with chitosan containing cinnamon and lemon grass essential oil at a refrigerated temperature had lower lipid oxidation, and thus had a longer shelf life than control fillets. In a study by Cardoso et al. (2017) the results showed that the active films poly (butylene adipate co-terephthalate)–PBAT incorporated

Table 3 Sensory evaluation in different treatments during storage in refrigerator (4 ± 1 °C)

Treatment	Storage days					
	0	5	10	15	20	25
<i>Color</i>						
Control	9.78 $\pm$ 0.39 ^{a*}	8.00 $\pm$ 0.28 ^a	6.29 $\pm$ 0.39 ^a	4.42 $\pm$ 0.60 ^a	3.93 $\pm$ 0.53 ^a	2.36 $\pm$ 0.55 ^a
2% SE	9.57 $\pm$ 0.45 ^a	8.07 $\pm$ 0.35 ^a	6.43 $\pm$ 0.61 ^a	5.00 $\pm$ 0.29 ^a	4.35 $\pm$ 0.69 ^{ab}	2.50 $\pm$ 0.50 ^a
4% SE	9.78 $\pm$ 0.39 ^a	8.21 $\pm$ 0.64 ^a	7.07 $\pm$ 0.3 ^{ab}	6.07 $\pm$ 0.35 ^b	4.71 $\pm$ 0.39 ^b	3.92 $\pm$ 0.53 ^b
6% SE	9.78 $\pm$ 0.39 ^a	8.14 $\pm$ 0.80 ^a	7.21 $\pm$ 0.27 ^b	6.21 $\pm$ 0.39 ^b	6.00 $\pm$ 0.28 ^c	4.28 $\pm$ 0.57 ^b
<i>Odor</i>						
Control	9.78 $\pm$ 0.27 ^a	7.85 $\pm$ 0.48 ^a	6.37 $\pm$ 0.53 ^a	4.07 $\pm$ 0.35 ^a	2.55 $\pm$ 0.51 ^a	2.07 $\pm$ 0.12 ^a
2% SE	9.50 $\pm$ 0.41 ^a	8.00 $\pm$ 0.58 ^a	7.10 $\pm$ 0.19 ^b	5.88 $\pm$ 0.27 ^b	4.28 $\pm$ 0.57 ^b	2.54 $\pm$ 0.46 ^a
4% SE	9.35 $\pm$ 0.38 ^a	7.41 $\pm$ 0.34 ^{ab}	7.25 $\pm$ 0.25 ^b	5.92 $\pm$ 0.34 ^b	4.57 $\pm$ 0.53 ^b	3.81 $\pm$ 0.44 ^b
6% SE	9.21 $\pm$ 0.26 ^b	7.14 $\pm$ 0.24 ^b	7.28 $\pm$ 0.27 ^b	6.35 $\pm$ 0.47 ^{bc}	5.67 $\pm$ 0.42 ^c	4.49 $\pm$ 0.50 ^c
<i>Texture</i>						
Control	9.79 $\pm$ 0.27 ^a	7.78 $\pm$ 0.49 ^a	6.47 $\pm$ 0.47 ^a	4.04 $\pm$ 0.36 ^a	3.14 $\pm$ 0.47 ^a	2.04 $\pm$ 0.08 ^a
2% SE	9.79 $\pm$ 0.27 ^a	7.81 $\pm$ 0.51 ^a	7.18 $\pm$ 0.24 ^b	5.61 $\pm$ 0.27 ^b	4.25 $\pm$ 0.53 ^b	2.38 $\pm$ 0.32 ^a
4% SE	9.79 $\pm$ 0.27 ^a	7.86 $\pm$ 0.56 ^a	7.20 $\pm$ 0.23 ^b	5.95 $\pm$ 0.36 ^b	5.16 $\pm$ 0.49 ^c	3.76 $\pm$ 0.43 ^b
6% SE	9.79 $\pm$ 0.27 ^a	7.89 $\pm$ 0.57 ^a	7.32 $\pm$ 0.26 ^b	6.52 $\pm$ 0.37 ^c	6.00 $\pm$ 0.41 ^d	4.44 $\pm$ 0.52 ^c
<i>Overall acceptance</i>						
Control	9.36 $\pm$ 0.69 ^a	7.71 $\pm$ 0.49 ^a	5.40 $\pm$ 0.34 ^a	3.75 $\pm$ 0.25 ^a	2.86 $\pm$ 0.37 ^a	2.16 $\pm$ 0.19 ^a
2% SE	9.29 $\pm$ 0.76 ^a	7.99 $\pm$ 0.55 ^{ab}	6.14 $\pm$ 0.19 ^b	4.91 $\pm$ 0.30 ^b	4.05 $\pm$ 0.59 ^b	3.13 $\pm$ 0.47 ^a
4% SE	9.08 $\pm$ 0.72 ^a	8.35 $\pm$ 0.25 ^b	7.61 $\pm$ 0.57 ^c	6.61 $\pm$ 0.29 ^c	6.01 $\pm$ 0.48 ^c	4.34 $\pm$ 0.13 ^b
6% SE	9.12 $\pm$ 0.71 ^a	8.42 $\pm$ 0.20 ^b	7.87 $\pm$ 0.58 ^c	7.31 $\pm$ 0.24 ^d	6.00 $\pm$ 0.41 ^c	5.14 $\pm$ 0.45 ^b

*Different lower case letters in a column indicate significant differences between treatments ($p < 0.05$)

with oregano essential oil reduced the total microbial load of the Coliforms, *Staphylococcus aureus*, and psychrophile bacteria in fish fillets that play a role in fish storage and processing. In another study by Raeisi et al., the results showed that *Allium paradoxum* and *Eryngium caucasicum* extracts on silver carp in refrigerator conditions (4 ± 1 °C) reduced the microbial load of fillets (Raeisi et al., 2016). The combination of hydro ethanolic extract of *S. officinalis* L. protects against microbial and chemical growth. According to the results of the present study, the shelf life of fish fillets was increased using an extract at a temperature of 4 ± 1 °C. According to the results of chemical, microbial and sensory evaluations, the extract 6% was more effective in improving the shelf life of fish fillets.

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