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Research Article

Effect of aqueous extract of Iranian Sumac (*Rhus criaria* L.) on the shelf life of *Hypophthalmichthys molitrix* fillet during storage at 4°C

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Abstract

Sumac (*Rhus coriaria* L.), belonging to the Anacardiaceae family, grows in Mediterranean countries, North Africa, South Europe, Afghanistan and Iran. The sumac berries contain biological activities such as antioxidant, antimicrobial, hypoglycemic and antidiabetic. The present study examined the effects of water extract of sumac on the chemical, microbiological and sensory quality on silver carp (*Hypophthalmichthys molitrix*) fillets during storage at 4°C. Treatments in the present study included the following: Control without extract of sumac, 1, 2.5 and 5 % (w/v) the water extract of sumac. After 18 days of storage, the results showed that the lowest total viable count (TVC) (7.34 log 10 cfu g^{-1}), psychrophilic total counts (PTC) (7.21 log 10 cfu g^{-1}), pH (6.43), total volatile basic nitrogen (TVB-N) (23.38 mg N $100g^{-1}$), Thiobarbituric acid (TBA) (1.66 mg MDA kg^{-1}) and peroxide value (PV) (5.28 meq kg^{-1}) were recorded in 5% sumac water extract treated samples, while the highest TVC (9.01 log 10 cfu g^{-1}), PTC (8.97 log 10 cfu g^{-1}), pH (7.15), TVB-N (38.71 mg N $100g^{-1}$), TBA (2.82 mg MDA kg^{-1}) and PV (9.41 mEq kg^{-1}) occurred in the control group. The best outcome of sensory evaluation was acquired in samples that treated with 5% sumac water extract and was significantly different ($p \leq 0/05$), when compared to the control group after 18 days of storage. Natural preservatives such as sumac extract can be used as a safe method for storage of silver carp fillets, which is quite promising for food industry.

Keywords: *Rhus criaria*, *Hypophthalmichthys molitrix*, Shelf life, Fillet, Total viable count, Psychrophilic total counts.

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Introduction

Fish is considered as an important part of a healthy diet and an excellent source of protein, essential minerals, trace elements and vitamins (Perez-Alson *et al.,* 2003). Fresh fish are highly perishable products due to their biological composition. Under normal refrigerated storage conditions, the shelf life of these products is limited by enzymatic and microbiological spoilage (Siyertsvik *et al.,* 2002). Fish is preserved when the basic causes of its spoilage are controlled. The methods for preserving food are varied and, depending upon their basic approach, maybe effective for either short or long periods of spoilage. Preservation of high moisture-fresh food like fish may be accomplished by low temperature, but just for a short time (Ghaly *et al.,* 2010; Tosi *et al.,* 2007).

Increasingly, consumers are demanding more natural, minimally processed products. Increasing awareness of the consumers for the use of synthetic preservatives needs research for more efficient antimicrobials and antioxidants with fewer side effects on human health[4]. The extracts contain bioactive phenolic compounds which have recently been recognized for their efficacy in providing significant antioxidant activity to human foods (Gibis *et al.,* 2012; Rosales Soto *et al.,* 2012). The use of various combined preserving methods and substances is under consideration. Polyphenols from various natural sources include plants, sumac and other sources; have been reported to have a variety of biological effects, including antimicrobial activities (Aliyazicioglu *et al.,* 2013; Gulcin *et al.,* 2010).

Sumac (*Rhus coriaria* L.), belonging to the Anacardiaceae family, is a small tree or shrub. It grows in Mediterranean countries, North Africa, South Europe, Afghanistan and Iran (Kosar *et al.,* 2007). The sumac berries contain flavones, tannins, anthocyanins and organic acids which provide antioxidant and antimicrobial activity. Several studies (Kosar *et al.,* 2007; Naser-abbas *et al.,* 2004) demonstrated that sumac berries contain biological activities such as antioxidant, antimicrobial, hypoglycemic and antidiabetic. As free radical, the phenolic compounds can potentially interact with biological systems and prevent the human neurodegenerative diseases, cardiovascular disorders and cancer (Poudel *et al.,* 2008). Development of natural preservative with high antioxidant, antibacterial activities which prolong the shelf life of fish and fish products is desirable. The aim of the present study was to determine the antimicrobial and antioxidant effect of sumac on silver carp fresh fillets. Chemical, microbiological and sensory analyses were measured to investigate the quality changes and to determine the shelf life of silver carp fresh fillets during storage at 4°C.

Materials and methods

All experiments were performed in the Laboratory of Food Hygiene, Veterinary Faculty in University of Zabol.

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Extraction

Fresh sumac fruit was prepared from Tabriz (Iran) and transferred to the laboratory. Sumac fruit was dried at 45 °C for 72 h and milled to a particle size less than 0.5 mm. To prepare a water extract, five grams of sumac was soaked in 95 ml distilled water for 1 h at room temperature with occasional stirring followed by gentle boiling for 2 min on a plate heater equipped with magnetic stirrer. The extract was obtained by cooling and filtration through whatman 4 filter paper and then autoclaved (Egan *et al.*, 1997).

Sample preparation

Silver carp with an average weight of 800±10 g were purchased at local markets and transferred to the laboratory. Fish samples were placed in ice boxes and transferred to the laboratory. Immediately after delivery, whole fish were filleted (100±10 g each) manually. Clean fish fillets were separated into four groups. Control sample, without added water extract of sumac and treated samples that dipping in water extract of sumac 1, 2.5 and 5% (w/v), packed in sterile (gamma irradiated) plastic bag. The samples stored in chill room (4°C) and used for analysis on 0, 3, 6, 9, 12, 15 and 18 days. The experiments were performed in three replicates.

Bacteriological analysis

For total viable counts (TVC) and psychrophilic total count (PTC) tests, fish muscle (10 g) of each treatment was aseptically weighed and homogenized with 90 ml sterile 0.85% normal saline for 1 min at room temperature. The decimal dilutions were prepared and then 0.1 ml of each dilution was pipetted onto the surface of Tryptic Soy Agar (TSA). They were incubated for 48 h at 35°C for TVC according to Egan *et al.* (1997) and 10 days at 4°C for PTC based on McMeekin *et al*. (1993). Microbial loads were expressed as log 10 cfu g^{-1}.

Chemical analysis

pH determination

The pH value was recorded using a pH meter (Multiline P4, WTW). Five grams of fish sample was homogenized thoroughly with 45 ml of distilled water for 30 sec and homogenated solution was used for pH determination.

Total volatile basic nitrogen determination

The TVB-N was estimated using steam distillation method (AOAC, 2005). The steam distillation was carried out by distillation after the addition of magnesium oxide (MgO) to the homogenized fish collected in a flask containing 2% aqueous solution of boric acid H_3BO_3 and a mixed indicator produced from dissolution of 0.1 g of methyl red to 100 ml of ethanol (ethyl alcohol). Then, the boric acid solution was titrated with 0.1 N sulfuric acid solution. The TVB-N value (mg N $100g^{-1}$ fish flesh) was determined based on the consumption of sulfuric acid.

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Peroxide value determination

The peroxide content in the total lipid extract was determined according to the method of AOAC (AOAC, 2005). Results were expressed as mEq O_2 kg^{-1} lipid.

Thiobarbituric acid determination

The TBA value (as malondialdehyde) was determined calorimetrically. A mixture of 200 mg sample and 1 ml of 1-Butanol for dissolving was added to 25 ml volumetric flask. Five milliliters of the mixture were pipetted into a dry stoppered test tube, and 5 ml of TBA reagent (prepared by dissolving 200 mg of 2- TBA in 100 ml 1-Butanol, filtered, stored at 4°C for not more than seven days) was added. The test tubes were stoppered, vortexed, placed in a water bath at 95 °C for 120 min, and then cooled. Absorbance (As) was measured at 530 nm against water blank. A reagent blank was run and absorbance (Ab) was recorded. TBA value (mg malondialdehyde kg^{-1}) was obtained using this formula (Mcmeekin *et al.*, 1993):

TBA= As –Ab×50/200

Sensory evaluation

Sensory quality of fish sample was evaluated by ten trained panelists. The fillets samples were fried (sunflower oil, Iran) individually in a Grill machine (Tefal, France) for approximately 3 minutes at 180 °C. The panelists have scored for color, odor, flavor, overall acceptability and texture, using a five-point hedonic scale (1, extremely dislike to 5, extremely like) (ASTM, 1969):

Statistical analysis

Data were analyzed using One-Way ANOVA by SPSS for Windows, version 16 (SPSS, Chicago, IL, USA). When differences were significant (p<0.05), the mean values were evaluated by Duncan test. Kruskal-Wallis test was used for sensory evaluation. The Mann-Whitney U-test was used to Paired comparisons test.

Results

The major findings of this study showed that samples treated with 5% sumac water extract had the best sensory evaluation score, and were significantly different (p≤0/05), when compared to the control group after 18 days of storage.

Microbiological analysis

TVC and PTC of silver carp fillets during chill storage are shown in Fig. 1 a-b. Total counts in the fishery products are useful tool for quality evaluation of shelf-life and post-processing contamination, while psychrotrophic bacteria are particularly the major group of microorganisms responsible for spoilage of fresh seafood (Bensid *et al.*, 2014; Huss, 1995). The initial TVC value of silver carp was 4.16, 4.13, 4.07 and 3.93 log 10 CFU g^{-1} for 0, 1, 2.5 and 5%, respectively, indicates good quality of fish. The alternations in TVC during 18 days of storage showed that microbial growth had been significantly (p<0.05) influenced by the addition of sumac extract. 0, 1, 2.5 and 5% fish

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fillets samples exceeded the value of 7 log cfu g^{-1} for TVC, considered as the upper acceptability limit for fresh marine species (Mahmoud *et al.*, 2004) on days 12, 12, 15 and 18 of storage, respectively (Fig. 1a). Results of TVC showed that the sumac extract, especially its phenolic components, has antimicrobial activity (Ahn *et al.*, 2007; Campos *et al.*, 2014).

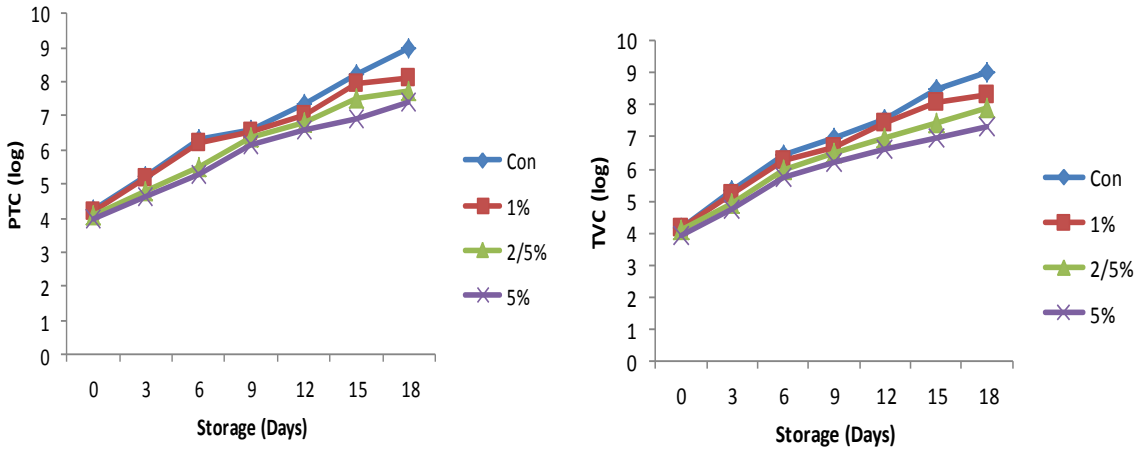


Figure 1: Changes in TVC (Total Viable Counts) (A), PTC (Psychrophilic Total Counts) (B) values of silver carp fillets during storage at 4C. control, 1, 2.5 and 5% (w/v) water extract of sumac.

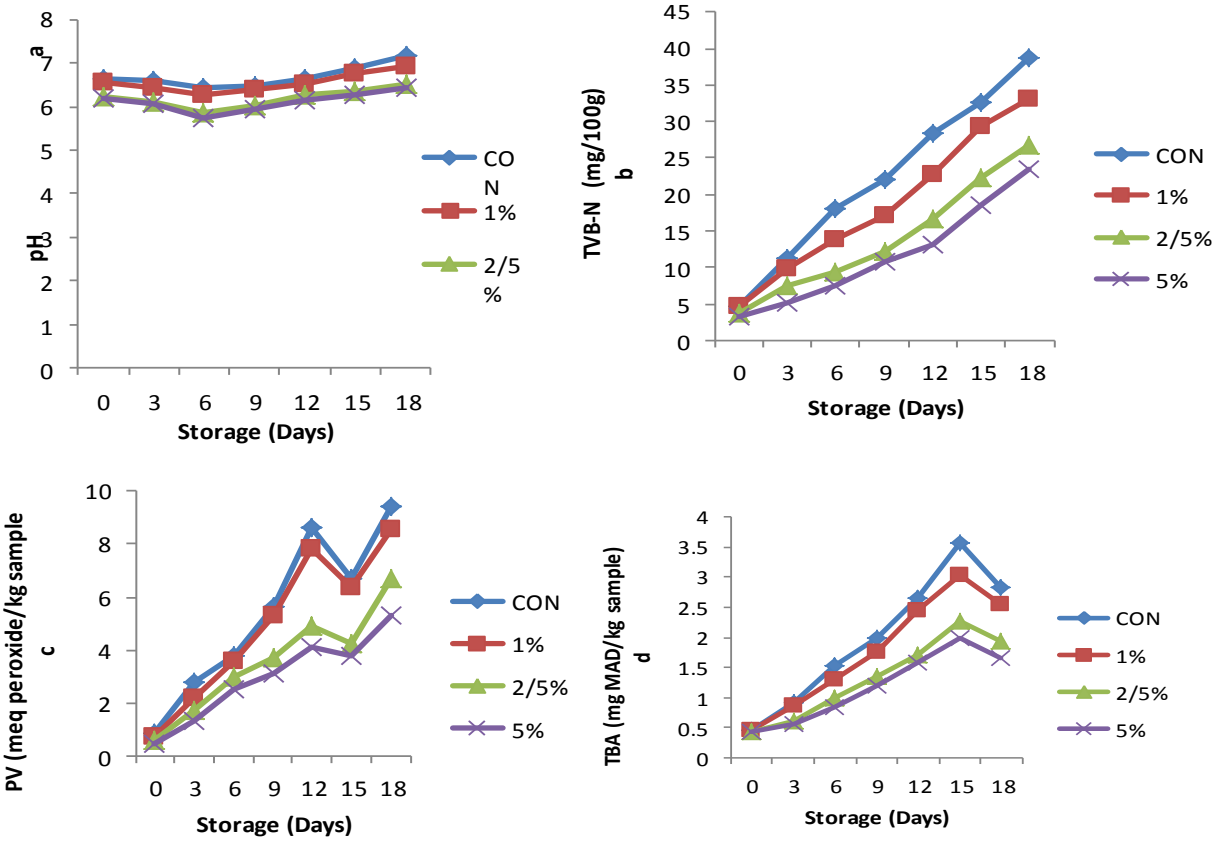


Figure 2: Changes in pH (A), TVBN (total volatile basic nitrogen) (B), PV (peroxide value) (C) and TBA (Thiobarbituric Acid) (D) values of silver carp fillets during storage at 4°C. Control, 1, 2.5 and 5% (w/v) water extract of sumac.

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The psychrotrophic bacteria are the major group of microorganisms responsible for spoilage of aerobically stored fresh fish at chilled temperatures (Gram and Daglaard, 2002). The initial PTC value of silver carp was 4.14, 4.09, 4.01 and 3.85 Log 10 CFU g^{-1} for 0, 1, 2.5 and 5%, respectively. In control and P1 group samples psychrotrophic bacteria counts exceeded the value of 7 log 10 CFU g^{-1}, on the 12th storage day. On the other hand, in P2 and P3 group samples psechrotropic bacteria counts exhibited a growth under the 7 log CFU g^{-1} on the 15th and 18th storage days, respectively. Statistically significant differences were found between the samples with respect to the storage duration (p<0.05).

Chemical analysis

pH value

The changes in pH of silver carp fillets as a function of treatments and storage time are shown in Fig. 2a. Significant pH changes can be seen between treatments and storage times. pH value of the fillet usually ranges from 5.73 to 7.15. The pH of fresh fish is close to the neutral pH, but after death the lactic acid is formed which firstly falls and then rises again with spoilage. The initial pH of control on day 0 was 6.64 indicating the freshness of fish samples. All samples showed an increased pH value with extended storage period (Fig. 2a). Significant differences were found between the samples (p<0.05). At the end of storage time, the pH values of the samples in the present study reached maximum levels of 7.15, 6.92, 6.51 and 6.43 for control, 1, 2.5 and 5%, respectively.

Total volatile basic nitrogen value

TVB-N is one of the most widely used indices of seafood quality and is associated with the amino acid decarboxylase activity of microorganisms during storage (Poudel *et al.*, 2008). The changes in TVB-N of silver carp fillets as a function of treatment and storage time are shown in Fig. 2b. The results show that the TVB-N values increase significantly (p<0.05) during the storage at 4 ˚C. The total phenolic also had a significant effect on all the TVB-N values (p<0.05). The lowest values were found on the first day of storage for the 2.5 and 5% total phenolic and the highest were found on the 18 days of storage for control group. The significant differences were observed between all treatments for all days except the first day of storage. The initial TVB-N values in fillet were determined as 4.71, 4.71, 3.77 and 3.32 (mg N $100g^{-1}$ flesh) for 0, 1, 2.5 and 5%, respectively and increased with time of storage in all groups. Its increase is related to the activity of spoilage bacteria and endogenous enzymes because enzymes are still active (Gulcin *et al.*, 2010).

Peroxide value

The PV values of the samples are shown in Fig. 2c. The results showed that the PV values increase significantly (p<0.05) during storage at 4 ˚C. The total phenolic also had a significant effect on all the PV values (p<0.05). Significant lower (p<0.05) PV value

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was observed for treated samples during the storage period at 4˚C. The PV of silver carp fillets was modified during refrigerated storage. The initial PV values in fillet were determined as 0.87, 0.7, 0.56 and 0.46 (mEq O_2 kg^{-1} lipid) for 0, 1, 2.5 and 5%, respectively and increased with storage time in all groups. There were no significant differences among all treatments on the first day; however, a significant difference in PV was noticed between the control and 1, 2.5 and 5% sumac extracts during the storage (p<0.05). The PV values of the 1, 2/5 and 5% sumac extracts were lower than the control. Significant differences were found between the samples (p<0.05). At the end of the storage time, PV values of the samples in the present study reached maximum levels of 9.41, 8.58, 6.71 and 5.28 (mEq O_2 Kg^{-1} lipid) for 0, 1, 2.5 and 5%, respectively.

Thiobarbituric acid value

The evaluation of TBA values of the silver carp fillets during refrigerated storage are shown in Fig. 2d. The results show that TBA values increase significantly (p<0.05) during the storage at 4˚C. The lowest values were found on the first day of storage for the 2.5 and 5% total phenolic and the highest were found on the 18 days of storage for control group. The significant differences were observed between all treatments for all days except first day of storage. In the present study, the initial TBA value of silver carp was 0.46, 0.45, 0.43 and 0.43 mg MDA kg^{-1} for 0, 1, 2.5 and 5%, respectively. On the first day, the sumac extract had a significant effect on the TBA values of 1, 2.5 and 5% treatments in comparison with the control.

Sensory analysis

The results of the sensory evaluation are given in Table 1. Acceptability scores for flavor, odor, texture, color and overall acceptability of silver carp fillet samples treated by the sumac, decreased significantly (p<0.05) with time of storage. The results indicate the sensory scores showed a significant decline in all samples with increasing storage period, and the results also indicated that the sensory evaluation values decreased with increasing chemical and microbial spoilage (p<0.05).

Table 1: Effect of aqueous extract of sumac on the sensory properties of silver carp fillet during storage at 4˚C.

Storage time (Days)		Flavor	Odor	Texture	Color	Overall acceptability
0	Control	4.5±0.57^{a}	4.6±0.51^{a}	4.8±0.42^{a}	4.8±0.42^{b}	4.4±0.51^{a}
	1%	4.5±0.57^{a}	4.6±0.51^{a}	4.8±0.42^{a}	4.3±0.67^{ab}	4.5±0.52^{a}
	2.5%	4.7±0.48^{a}	4.6±0.51^{a}	4.9±0.31^{a}	3.9±0.99^{a}	4.5±0.52^{a}
	5%	4.7±0.48^{a}	4.6±0.51^{a}	5±0.00^{a}	3.6±1.07^{a}	4.6±0.51
3	Control	3.7 ±0.48^{a}	4.1±0.73^{a}	4±0.66^{a}	4.3±0.48^{b}	3.6±0.51^{a}
	1%	3.8±0.42^{a}	4.3±0.48^{a}	4.3±0.67^{a}	4.1±0.73^{b}	3.9±0.73^{a}
	2.5%	4.5±0.52^{b}	4.4±0.51^{a}	4.5±0.52^{a}	3.7±0.94^{ab}	4.1±0.56^{ab}
	5%	4.6±0.51^{b}	4.5±0.52^{a}	4.5±0.52^{a}	3.3±0.82^{a}	4.5±0.52^{b}
6	Control	3.1±0.73^{a}	2.9±0.73^{a}	3.1±0.73^{a}	3.4±0.51^{a}	2.5±0.52^{a}
	1%	2.4±0.69^{a}	3.1±0.73^{ab}	3.6±0.51^{a}	3.3±0.78^{a}	3.3±0.67^{b}
	2.5%	3.1±0.56^{b}	3.6±0.51^{b}	3.9±0.66^{b}	3.2±0.78^{a}	3.4±0.51^{b}
	5%	3.3±0.67^{b}	3.6±0.51^{b}	4±0.66^{b}	3±0.81^{a}	4.1±0.56^{c}

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Table 1 continued:

	Control	2.1±0.73[a]	1.7±0.48[a]	2.1±0.73[a]	2.5±0.52[a]	1.4±0.51[a]
9	1%	2.4±0.69[a]	2.4±0.51[b]	2.3±0.48[a]	2.6±0.51[a]	2.3±0.48[b]
	2.5%	3.1±0.56[b]	2.7±0.48[b]	3±0.66[b]	2.7±0.48[a]	3±0.66[c]
	5%	3.3±0.67[b]	3±0.66[c]	3.3±0.48[b]	2.7±0.48[a]	3.6±0.51[c]
	Control	-	-	-	-	-
12	1%	-	-	-	-	-
	2.5%	2.2±0.63[a]	2.1±0.56[a]	2.4±0.51[a]	1.4±0.51[a]	1.5±0.52[a]
	5%	2.7±0.48[a]	2.3±0.48[a]	2.7±0.48[a]	1.7±0.48[a]	1.7±0.48[a]
	Control	-	-	-	-	-
15	1%	-	-	-	-	-
	2.5%	-	-	-	-	-
	5%		1.7±0.48	1.7±0.48	1±0.00	1.2±0.42

Values are mean±SD. Different superscripts in the same column denote the significant difference (p<0.05)

Spoilage and quality deterioration can be assessed by chemical and physical methods and sensory evaluation [31]. Not all chemical assessments give good correlation to quality changes; hence sensory evaluation is a necessity (ICMSF, 1986).

The sensory properties of the control group were received a lower score than the 2.5% and 5% groups. This result showed that the acceptable limits of microbial parameters is not confirmed the quality changes, thus the sensory evaluation is requirement.

Discussion

PV is one of the important indices of meat spoilage (Olafsdottir *et al.,* 1997). It indicates the existence of peroxides and hydroperoxides concentrations that are produced during the early stages of lipid oxidation. Lower PV values in the samples treated with sumac extract might be linked with phenolic content (Huss, 1995). The use of antioxidants was very effective in reducing lipid oxidation in fish fillets because of those phenolic compounds which act as inhibitors for radical reactions on autoxidation (Lean and Mohamed, 1999). Ojagh *et al.* (2010) reported that the PVs increased in rainbow trout during storage at refrigerator, but this increase was lower in samples that treated with chitosan and cinnamon due to their antioxidant activity. In present study, PV increased during the 12th day of storage. After this, a sudden decrease in all treatments was observed (Fig. 2c), which may be related to secondary oxidation products and volatile compounds (Barriuso *et al.,* 2013). The PV of fillets increased between the 15th and 18th day of storage (Fig. 2c). These results were in agreement with the finding of Ozogul *et al*. (2006) on Anguilla *anguilla.*

In the present study, the initial TVC value of silver carp was 4.16, 4.13, 4.07 and 3.93 log 10 CFU g^{-1} for 0, 1, 2.5 and 5%, respectively, indicates good fish quality, which is in agreement with results (4.6 log CFU g^{-1}) reported by Mahmoud *et al.* (2004) for fresh carp.

TVB-N is one of the most widely used indices of seafood quality and is associated with the amino acid decarboxylase activity of microorganisms during storage (Bensid *et al.,* 2014). TBA index is a widely used indicator for the assessment of degree of lipid oxidation (Kosar *et al.,* 2007). At the end of storage time, the TVB-N values of the samples in the

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present study reached to the maximum levels of 38.71, 33.2, 26.69 and 23.38 for 0, 1, 2.5 and 5%, respectively. Samples treated with 5% had the lowest levels of TVB-N compared to the other samples. This finding could be due to the antimicrobial activity of the sumac and the reduction of the capacity of the bacteria to carry out oxidative deamination of non-protein nitrogen compounds (Fan *et al.,* 2009). Similar TVB-N values were reported by Ali *et al*. (2010). A level of 25 mg N $100g^{-1}$ TVB-N has been considered the upper limit above which fishery products are considered spoiled and unfit for human consumption (Kilincceker *et al.,* 2009; Harpaz *et al.,* 2003). Significant differences were found between the samples ($p<0.05$).

pH is an important and effective indicator of meat quality (Suvanich *et al.,* 2000). The lower pH observed in the samples treated with sumac extract can be attributed to its antibacterial properties (Bayder *et al.,* 2004). Phenolic compounds of sumac extract can increase microbial inhibition, protect fillets against internal protease, and inhibit protein breakdown and amine production (Harpez *et al.,* 2003). The increases of pH values during the storage period may be attributed to the accumulation of alkaline compounds, such as ammonia common compounds and trimethylamine, mainly derived from microbial action (Manju *et al.,* 2007).

Determination of TBA is based on the measurement of malonaldehyde determining the secondary oxidation products related to spoilage of fish[35] which are the initial products created by the reaction of polyunsaturated fatty acids with oxygen. The decrease of TBA in the treatments could be due to the effects of antioxidants in reducing peroxide[23] value showed a general and gradual increase with the storage time for all groups. Increases in TBA values in different samples indicated oxidation in the silver carp fillets during refrigerated storage. This was probably due to the destruction of hydroperoxides into secondary oxidation products, specially aldehydes in the later stages of lipid oxidation (Al-bandak *et al.,* 2009). Similarly, Tajik *et al*. (2014) observed strong antioxidant effects of clove and grape seed extracts, and reported that the antioxidant potential of this extract significantly restrained TBA values in silver carp fillets. At the end of storage time, the TBA values of the samples in the present study reached maximum levels of 2.82, 2.54, 1.93 and 1.66 mg MDA kg^{-1} for 0, 1, 2.5 and 5%, respectively. The lowest increase in TBA value was observed in the 5% treatment. According to Goulas and Kontominas (2007), the TBA value of 1-2 mg MDA kg^{-1} in fish is usually regarded as the limit beyond which fish will normally develop an objectionable odor/test. This effect may be related to the presence of flavonoid in the sumac because flavonoids have an antioxidant effects.

Fan *et al.* (2009) reported that sensory scores of silver carp reduced with increasing storage period in compared to those of treated with tea polyphenols. It is well known that fish spoilage gives rise to the subsequent

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development of strongly fishery, rancid and putrid odors, and fish are clearly rejected for consumption by any taste panel. Based on the results, 5% treatment could be retaining their good quality in terms of sensory evaluation compared to the other treatments. These results are agreed with the results of microbial and chemical analyses.

These results showed that samples with high extract of sumac have acceptable overall scores, due to the limiting effect of sumac on microbiological and chemical activities. Five percent level added to the samples were assessed as the most acceptable products by the panelists. Natural preservatives such as sumac extract can be used as a safe method for storage of silver carp fillets, which is quite promising for food industry.

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