Use of pomegranate peel and artichoke leaf extracts to improve the quality of marinated sardine (*Sardinella aurita*) fillets

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Revised: 24 July 2019 / Accepted: 9 September 2019 / Published online: 13 September 2019
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Abstract Effects of pomegranate peel and artichoke leaf aqueous extracts on biochemical, microbiological and sensorial quality of sardine fillets was investigated during marination and storage for 90 days. Sardine fillets were marinated with 4% acetic acid, 10% NaCl and either 5% pomegranate peel extracts or 5% artichoke leaf extracts. The control sample was marinated with only 4% acetic acid and 10% NaCl. The antimicrobial activity of pomegranate peel and artichoke leaf extracts led to reduce total viable counts and total coliform during marination and storage. Samples marinated with pomegranate peel and artichoke leaves showed better oxidative stability and higher content of polyunsaturated fatty acids. Higher values of free fatty acids and histamine were found in control samples, whereas adding extracts of pomegranate peel or artichoke leaves significantly decreased total volatile basic nitrogen and trimethylamine during storage. Greater color and appearance scores were found for samples marinated with pomegranate peel and artichoke leaf extracts than the control samples.

Keywords Marinated sardine · Vegetable byproducts · Aqueous extracts · Antioxidant activity · Antimicrobial activity

Introduction

Sardinella aurita (Clupeidae, Teleostei) is one of the most important species in the Mediterranean Sea and one of the most common fish species in Tunisia (Ennouri et al. 2013). Its high protein and polyunsaturated fatty acid content makes it beneficial for human health. The effect of these fatty acids is well documented in numerous investigations (Ennouri et al. 2013; Zlatanov and Laskaridis 2007). It has a low commercial value, and is generally consumed fresh or canned. However, marinated products are usually appreciated and are increasingly part of Tunisian culinary products. Marinated fish are consumed with no heat treatment and thus has a limited shelf life (Gokoglu et al. 2009). The main objectives of the marination process are to tenderize and improve flavor and to inhibit microbial growth (Sallam et al. 2007). Sardines are especially suited to marination because of their fat content (Kilinc and Cakli 2004). However, polyunsaturated fatty acids are subject to oxidation which is responsible for a rancid flavor and a decrease in nutritional value (Topuz et al. 2014). The quality of marinated fish changes according to the initial quality of fish, the conditions of marination and storage and the use of preserving substances (Demirok et al. 2014; Gokoglu et al. 2009; Topuz et al. 2016). Various synthetic antioxidants have been used in food products to prevent lipid oxidation but there is a growing demand for naturally derived antioxidants obtained from a wide variety of plant sources. In Tunisia, increasing attention has been given to use fruits or vegetables processing byproducts as functional food ingredients since they are rich in beneficial bioactive compounds. Among these types of byproducts, two are of interest in this work: pomegranate peel and artichoke leaves.

The pomegranate plant (*Punica granatum* L.) is native to the Mediterranean region. Peel and peel extracts are a

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rich source of polyphenols, including ellagitannins, galloylannins, ellagic acids, gallagic acids, catechins, anthocyanins, ferulic acids and quercetins (Akhtar et al. 2015; El-Said et al. 2014; Topuz et al. 2014). These phytochemicals exhibit significant free radical scavenging and anti-microbial properties and are reported to have beneficial effects against many critical diseases (Akhtar et al. 2015). Several studies have reported that the phenolic content of pomegranate peel was 10 times higher than that found in the pulp (Masci et al. 2016).

The globe artichoke (*Cynara cardunculus* L.) is a perennial plant cultivated in the Mediterranean region for its edible young flower heads (Piston et al. 2014). Artichoke leaves, which are unvalued byproducts of the canning industry, are rich in phenolic compounds and chlorogenic acid and the isomers 1, 3-,3,4 and 4,5-di-o-caffeoylquinic acid are the major bioactive compounds present in its phenolic composition, which have a strong antioxidant and antimicrobial capacity (Zhu et al. 2004).

Despite these excellent health benefits, the use of pomegranate peel and artichoke leaf extracts in food is still rare and underestimated. In this work, we aimed to use pomegranate peel and artichoke leaf extracts as ingredients in the pickling solution used to marinate sardine fillets. Their effects on microbiological, biochemical and sensory quality changes of sardine fillets during marination and storage have been investigated.

Materials and methods

Raw materials

A total of 30 kg of fresh sardines (*Sardinella aurita*) were purchased within one-day post capture from the fish market of Tunis. Catching of the fish was carried out in February. During this period of biological rest, the lipid content is high in the muscles (Ben Rebah et al. 2010). The fish were directly transferred to the laboratory in polystyrene boxes within 1 h after purchase. Sardines were immediately, eviscerated, filleted and washed.

Marination process

Optimization of marination time and acetic acid concentration

Acetic acid and NaCl were used for the marinade. Before starting this study, a preliminary trial based on a two-factor experience plan was run to optimize acetic acid concentration and immersion time. The high and low levels of acetic acid were 2 and 4%, respectively. Immersion times

were tested at 48 and 72 h. Responses were measured for pH and taste. Results showed that, 4% of acetic acid and 72 h of marination, gave the lowest pH value and the best taste (more appreciate by a panel). These parameters were then used for the marination process.

Preparation of pomegranate peel and artichoke aqueous extract

Pomegranates and artichokes were purchased from a local market. Pomegranate peel and artichoke leaves were manually removed and dried in an oven at 30 °C for 10 days (Akhtar et al. 2015). Dried pomegranate peel and artichoke leaves were then ground into a fine powder using food chopper (DPA241, Moulinex, France). Infusions were prepared as described by Piston et al. (2014). One liter of distilled water at 95 °C was added respectively to 20 g of the pomegranate powder and to 20 g of the artichoke powder. The liquid mixtures were left for 10 min than filtered through cotton. The extracts were stored at 4 °C until use.

Process

Sardine fillets were divided into 3 groups. The first group (CS) was marinated by soaking the fish fillets in a solution of 4% acetic acid and 10% NaCl, with a final pH of 2.1. The second and third groups were marinated in the same solution (4% acetic acid, 10% NaCl) with an addition of 5% artichoke extract (AS) and 5% pomegranate peel extract (PS), respectively (El-Said et al. 2014). The pH values of AS and PS solutions were 2.2 and 2.3, respectively. The ratio of fish:solution was 1:1.5. Immersion was done at ambient temperature (25 °C). After 72 h of marination, sardine fillets were removed from the solutions and put into glass jars filled with olive oil. A total of 12 glass jars were prepared per group. Packed sardines were stored at 4 °C for 90 days.

Analysis

Antioxidant activity

Antioxidant activity of the pomegranate peel and artichoke extracts was studied by evaluating the free radical scavenging effect on the 1,1-diphenyl-2-picrylhydrazine (DPPH) radical. The determination was based on the method described by El-Said et al. 2014. 100 µl of pomegranate peel and artichoke extracts were diluted (1:100) with methanol water (6:4) (v/v) and then mixed with 2 ml of 0.1 mM DPPH in methanol. The mixture was vigorously shaken, then allowed to stand in the dark at

room temperature for 30 min. Absorbance was measured at 517 nm by a spectrophotometer (6715 UV/Vis Spectrophotometer, Jenway). The absorbance value of the methanolic solution of DPPH was also measured at 517 nm. The reaction mixture without DPPH was used for background correction.

DPPH scavenging activity

$$= [1 - (\text{Abs sample 517 nm} / \text{Abs sample control 517 nm})] \times 100$$

Microbiological analysis

For the microbiological analysis, 10 g of each sample were collected aseptically and diluted with 90 ml of sterile peptone water (Accumix, Belgium) using a Stomacher 80 Biomaster. Serial 10-fold dilutions were prepared in sterile peptone water. Appropriate dilution samples (1 or 0.1 ml) were poured or spread in triplicate on different growth media. Total viable counts (TVC) and Lactic Acid Bacteria (LAB) were determined on Plate Count Agar (PCA) (Accumix, Belgium) and on MRS agar (Accumix, Belgium) respectively, after 48 h of incubation at 30 °C, coliforms on Desoxycholate Agar (Accumix, Belgium) after 24 h at 37 °C for total coliforms and 44 °C for fecal coliforms and *Staphylococcus aureus* on Baird Parker Agar (Accumix, Belgium) added with sodium tellurite and egg yolk (Essid and Hasouna 2013).

Chemical analysis

The pH of the sardine fillets was determined using a hand-held pH meter (Testo 206, pH2, France) equipped with a penetrating glass electrode. All measurements were performed at room temperature. The proximate chemical composition of sardine fillets was determined as crude protein ($N \times 6.25$), crude fat and moisture (AOAC 2000). Total volatile base nitrogen (TVB-N) and trimethylamine nitrogen (TMA-N) were determined by the micro-diffusion method of Conway (Cobb et al. 1973). Specifically, TMA-N was determined after the addition of formaldehyde. The sardine content in TVB-N and TMA-N was expressed as mg TVB-N per 100 g of sample and mg TMA-N per 100 g of sample. rPeroxide values were measured based on the standard AOCS method (2009). The peroxide value of fish oil is expressed as milliequivalents of peroxide per 1000 g of oil.

Determination of fatty acids

Free fatty acids were extracted according to the Soxhlet method (ISO 1996). Fatty acids were converted to fatty acid methyl esters before being analyzed by shaking off a

solution of 0.2 g of oil and 3 ml of hexane with 0.4 ml of 2 N methanolic potassium hydroxide. The free fatty acid composition was determined by means of gas chromatography coupled with mass spectrometry (GC–MS) after methyl esterification. The analysis was done by an Agilent chromatograph (Agilent 59 75 B) equipped with a split injector and flame ionization detector in an Agilent 19091S-933 column (1% phenyl-methyl-siloxane, 30 m $\times$ 250 mm $\times$ 0.25 mm). Injector and detector temperatures were 270 and 290 °C respectively, and the flow rate of the carrier gas (helium) was 1 ml/min. The oven temperature increased from 80 to 300 °C at a rate of 3 °C/min and 10 min at 300 °C. Individual free fatty acids were identified by comparing their retention times with individual standards. Free fatty acids were expressed as a percentage of the total fatty acid content.

Histamine analysis

The histamine content was determined using the colorimetric method described by Patange et al. (2005). The interaction between the imidazole ring and p-phenyldiazonium sulfonate was made the basis of a quantitative colorimetric method for estimation of histamine. Histamine was extracted from fish muscle as described by Patange et al. (2005). Fish muscle without skin (5 g) was homogenized with 20 ml of 0.85% NaCl by a speed blender. The obtained mix was centrifuged at 12,000 $\times g$ for 10 min at 4 °C. One ml of muscle extract was put into a glass-stoppered test tube and diluted to 2 ml with saline and 0.5 g of salt mixture (6.25 g of anhydrous sodium sulfate added to 1 g trisodium phosphate monohydrate). The tubes were stoppered and shaken thoroughly. Then, 2 ml of n-butanol were added and the tubes were shaken vigorously for 1 min and allowed to stand for 2 min, and then they were shaken briefly to break the protein gel. The tubes were shaken vigorously for another few seconds and then centrifuged at 3100 $\times g$ for 10 min. The upper butanol layer (only 1 ml) was transferred into a clean and dry test tube and evaporated to dryness in a stream of nitrogen. The residue was dissolved in 1 ml of distilled water and then reacted for 5 min with p-phenyl diazonium sulfanate reagent. The absorbance of the color produced was measured immediately after 5 min at 496 nm. Distilled water was used as blank. The concentration of histamine in the sample was obtained from the standard curve for the corresponding absorbance measured at 496 nm. The histamine concentration in the sample was estimated using the following formula:

$$\text{Histamine (mg/100 g)} = (A \times 2 \times 25 \times 100) / (5 \times 1000) \\ = A \text{ mg/100 g}$$

where A is the value of histamine obtained in 1 g/ml from the standard curve.

Sensory analysis

Sensory analysis of the marinated fillets was performed by a panel of 12 trained panelists using a scoring test. Panelist consisted of personal and students of Food Technologies department who had experience in evaluating seafood. Three training sessions were planned to familiarize the panelists with the descriptors and to harmonize their score by comparing each individual score with the panel's mean score. Sardine fillets were served to the panelists to evaluate the following sensory attributes: color, flavor, juiciness, tenderness, acid taste, odor and global appreciation. Each parameter in the sensorial analysis was evaluated on a scale of 1 to 9. The panelists had to judge on the basis of the perceived intensity (1- low intensity; 9- high intensity).

Statistical analysis

All measurements were done in triplicate. Data were statistically analyzed using a two-way ANOVA procedure of STATGRAPHICS Centurion (XV) 16.1.15 software (Manugistics Inc, Cambridge, MA). Duncan's multiple range test was used to determine any significant difference between mean values, and evaluations were based on a significance level of $P < 0.05$.

Results and discussion

Antioxidant activity

The DPPH radical scavenging capacity was used to determine the free radical scavenging activity of artichoke leaf and pomegranate peel extracts. The pomegranate peel extract showed an 88.78% inhibitory activity against DPPH. Results obtained here are comparable to those of Ashoush et al. (2013), who found that the radical-scavenging activity of pomegranate peel extracts against DPPH was 96.24%. The antioxidant activity of pomegranate peel could positively correlate with amounts of total phenolics and flavonoids present (Basiri et al. 2015). Many authors showed that adding pomegranate peel to foods extract increased phenolic, flavonoid and thiol concentration with a significant improvement of free radical scavenging stability features (Akhtar et al. 2015; Ashoush et al. 2013). Water as an environment friendly solvent has been found to be very efficient for extracting antioxidants from pomegranate (El-Said et al. 2014).

Artichoke leaf extract showed a 16.54% inhibitory activity against DPPH. Lutz et al. (2011) found higher antioxidant capacity (% inhibition of DPPH radical) of 30.5% whereas, El-Sayed et al. (2018) reported 20%. Piston et al. (2014) showed that an infusion of artichoke

leaves could be a potential source of natural antioxidants due to their high phenolic content.

Differences in the antioxidant activities of vegetable extracts reported in the literature, may be explained by the variety of vegetable species, the processing methods used, the type of the solvent, or solvent ratio, the particle size of the plant material and the solvent-solid ratio (Akhtar et al. 2015; Lutz et al. 2011).

Chemical composition of fresh sardine fillets

Moisture is the major component of the fish fillet and represents $74 \pm 2.12\%$ (g/100 g fresh fish). Sardine fillets also contain a high level of protein (18.1 ± 0.5) and fat ($6.99 \pm 0.04\%$). Similar results were reported by Boudhrioua et al. (2009). Variations in the chemical composition of sardine muscle depend on age, size, sex, environment, season, migratory swimming, feed intake, and sexual changes in connection with spawning (Ennouri et al. 2013).

Microbiological analysis

Microbiological results are given in Table 1. Total viable counts (TVC), total coliform counts (TC) and lactic acid bacteria (LAB) counts of fresh fillets were 3.07 log CFU/g, 1.87 log CFU/g, 2.39 log CFU/g, respectively. After marination, total viable and total coliform counts decreased, whereas LAB were inhibited. Kilinc et al. (2008) reported that activity of microorganisms is related to their adaptability to the marinade solution. It has been suggested that acid solution provides an environment suitable for the action of proteases present in the muscle. The products of proteolysis provide an energy source for the growth of acetic acid tolerant bacteria. Disappearance of LAB is related to high concentration of NaCl (10%). Kilinc and Cakli (2005) reported that to restrict LAB growth, a salt concentration no less than 6% and a low storage temperature is recommended for the marinating process. Unlike the results reported here, Kilinc and Cakli (2004), found a marked inhibition in the growth of TVC from 4.65 log CFU/g in raw sardine to < 10 CFU after marination; however, the marination solution used had a higher acetic acid and salt concentration, with in 7% and 14% respectively. Microbiological analysis revealed significant differences ($P < 0.05$) between control samples (CS) and those marinated with artichoke leaf (AS) and pomegranate peel (PS) extracts. Results obtained in the present study demonstrated antibacterial potential of pomegranate peel and artichoke leaf extracts. Many studies showed that pomegranate peel and artichoke leaf extracts revealed antimicrobial activity against bacteria (Basiri et al. 2015; Zhu et al. 2004). The possible mechanism of antimicrobial

Table 1 Changes in microbial flora of sardine fillets during marination and storage

log UFC/ g	FF	M72	Days of storage											
			30				60				90			
			CS	AS	PS	CS	AS	PS	CS	AS	PS	CS	AS	PS
TVC	3.07 ± 0.1	2.9 ± 0.09	2.1 ± 0.06	2.1 ± 0.09	1.8 ± 0.08	1.3 ± 0.12	1.4 ± 0.09	1.2 ± 0.05	1.02 ± 0.08	1.05 ± 0.09	0.5 ± 0.01	1.05 ± 0.08	0.5 ± 0.01	0.15 ± 0.01
TC	1.87 ± 0.09	1.65 ± 0.04	1.45 ± 0.05	1.3 ± 0.1	1.12 ± 0.09	1.03 ± 0.10	1.05 ± 0.08	–	–	–	–	–	–	–
LAB	2.39 ± 0.10	–	–	–	–	–	–	–	–	–	–	–	–	–
Mean value ± SD (n = 3)														

FF, fresh filets; M72, marination 72 h; CS, control samples; AS, samples marinated with artichoke leaf extracts; PS, samples marinated with peel pomegranate extracts; TVC, total viable counts; TC, total coliforms; LAB, lactic acid bacteria

effect of the extracts used in this study might be related to their phenolic compounds. These latter can bind to substrates such as minerals, vitamins and carbohydrates making them inaccessible for microorganisms, also they can denature enzymes. Furthermore, phenols can disturb the structure and function of the cell membrane (Shahidi and Nacz 2004). Moreover, the antimicrobial effects of pomegranate peel were more intense than those of artichoke leaves, since counts of TVC and TC were significantly lower in PS samples ($P < 0.05$). Phenolic contents differ among species, cultivars, age, growing conditions, harvest, post-harvest and storage conditions, and the technological procedures used for extraction (Tomas-Barberan and Espin 2001).

Marinated fillets are stored at 4 °C after having been bottled and covered with olive oil. Under these conditions, i.e. low temperature and lack of O₂ a significant decrease ($P < 0.05$) of microorganisms was observed during storage for all samples. In contrast, Karacam et al. (2002) reported a significant increase in the counts of TVC for anchovies brined in 14% NaCl solution and stored at 4 °C for 150 days. Difference in the microbial results among different studies could be related to the differences in the acetic acid and salt concentrations, the time and the storage condition for the marination process, the additives used and the initial microbial population (Sallam et al. 2007).

pH value

The initial pH of the fresh fillets was 5.97. After 24 h of marination, a considerable decrease was observed in pH, from 5.97 to 3.64, 3.41 and 3.69 for CS, AS and PS samples, respectively ($P < 0.05$) (Table 2). This decrease is due to the diffusion of acetic acid and salt into fish muscle during soaking. Similar results reported a sharp decrease in pH, by about 2–2.5 units, after marination with different concentrations of acetic acids and salt solutions (Gokoglu et al. 2004, 2009, Topuz et al. 2014). After 72 h of marination, pH values increased to reach values of 4.08; 3.65 and 3.79 ($P < 0.05$) for CS, AS and PS samples, respectively. This increase is related to the accumulation of alkaline compounds produced due to proteolysis and microbial growth. During storage, pH values for the three samples did not change significantly ($P > 0.05$) (Fig. 1a). At the end of the storage period the lowest pH was obtained for samples marinated with pomegranate peel extracts ($P < 0.05$) which showed the lowest counts of TVC. In marinated products, the pH value should not be more than 4.8 (Rehbein and Oehlschlager 1996). In this study, the pH of the three samples remained below 4.8. Kilinc et al. (2008) reported that pH value is not a criterion of spoilage, it has to be supported by other biochemical and sensorial analyses.

Table 2 Effect of pomegranate peel and artichoke leaf extracts on chemical parameters during marination

pH	ABVT (mg/100 g fish)			TMA(mg/100 g fish)			P value (meq/kg fish)		
	FF	M 24	M 72	FF	M 72	M24	FF	M 72	M 72
CS	5.97 ± 0.03 ^{a1}	3.64 ± 0.05 ^{a2}	4.08 ± 0.08 ^{a3}	4.48 ± 0.98 ^{a1}	4.03 ± 0.71 ^{a2}	3.98 ± 0.98 ^{a2}	0.74 ± 0.04 ^{a1}	0.87 ± 0.04 ^{a2}	0.89 ± 0.01 ^{a2}
AS	5.97 ± 0.03 ^{a1}	3.41 ± 0.04 ^{b2}	3.65 ± 0.09 ^{ab23}	4.48 ± 0.98 ^{a1}	3.89 ± 0.93 ^{b2}	4.02 ± 0.51 ^{b2}	0.74 ± 0.04 ^{a1}	0.81 ± 0.01 ^{a2}	0.83 ± 0.02 ^{a2}
PS	5.97 ± 0.03 ^{a1}	3.69 ± 0.05 ^{a2}	3.79 ± 0.03 ^{b3}	4.48 ± 0.98 ^{a1}	3.99 ± 0.35 ^{b2}	3.93 ± 0.62 ^{b2}	0.74 ± 0.04 ^{a1}	0.80 ± 0.02 ^{a2}	0.82 ± 0.04 ^{a2}
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Mean value ± SD (n = 3)

FF, fresh filllets; M24, marination 24 h; M72, marination 72 h; CS, control samples; AS, samples marinated with artichoke leaf extracts; PS, samples marinated with peel pomegranate extracts

Means with the same parameter and the same column with different superscript letters (a,b) are different ($P < 0.05$)Mean with the same parameter and the same line with different superscript numbers (1,2,3) are different ($P < 0.05$) **Fig. 1** Changes in pH (a), TVB-N (b), TMA-N (c) and PV (d) values of marinated sardine fillets during storage at 4 °C, CS, control sample; AS, samples marinated with artichoke leaf extracts; PS, samples marinated with peel pomegranate extracts

Total volatile base nitrogen (TVB-N)

TVB-N is produced from the degradation of proteins and non-protein nitrogenous compounds (Topuz et al. 2014). The initial TVB-N in raw fillets was 4.48 ± 0.98 mg/100 g fresh fillet. A significant decrease ($P < 0.05$) was observed in TVB-N values from 4.48 to 3.98, 4.02 and 3.93 mg/100 g fish fillet for CS, AS and PS samples, respectively, after the marinating process (Table 2). This decrease is related to the effects of acetic acid and salt which leached out the TVB-N during marination. Kilinc and Cakli (2004) reported a considerable decrease in TVB-N, from 10.3 to 6.5 after the marinating process of sardine fillets in a solution containing 7% acid acetic and 14% salt. During storage, TVB-N values ($P < 0.05$) gradually increased, for the three samples (Fig. 1b). This increase is maybe due to a combination of microbiological and autolytic degradation of nucleotides and free amino acids and microbial reduction of TMAO to TMA (Sallam et al. 2007). None of the samples obtained in this study, reached the limit (35 mg/100 g) during the storage period. The lower TVB-N content obtained in AS and PS samples compared to CS samples might be due to the inhibitory effects of phenolic compounds present in these extracts which acted against the spoilage bacteria. The lowest TVB-N values were found for samples marinated with pomegranate peel extract which showed the lowest number of TVC.

Trimethylamine content (TMA-N)

Fresh fish naturally contain trimethylamine oxide (TMAO). TMA-N results from the reduction of TMAO by bacterial and intrinsic enzyme activities which causes to the ammonia-like off-odor and fishy off-flavors (Gram and Huss 1996). A TMA value 10 mg/100 g sample was reported as the limit for acceptability of fish (Topuz et al. 2014). The TMA-N value of the raw material was found to be 0.74 ± 0.04 mg/100 g of fresh fillet. No significant differences ($P > 0.05$) in TMA-N values were observed during the marination process (Table 2). These results are similar to those of Sallam et al. (2007) who found that there were no significant differences in TMA-N values during the marination process of fish fillets in a solution containing 3% acetic acid and 12% salt. However, Kilinc and Cakli (2004) reported a slight increase in TMA values from 0.88 to 1.07 mg/100 g after marinating sardine fillets in acetic acid (7%) and salt (14%). TMA values of the three samples significantly increased during storage (Fig. 1c) to reach values of 2.63 ± 0.01 ; 1.946 ± 0.05 ; 1.905 ± 0.04 mg/100 g for CS, AS and PS, respectively ($P < 0.05$). Higher TMA-N values were found for CS samples compared to AS and PS samples. The lower TMA values in AS and PS samples may result from the inhibitory

effect of the pomegranate and artichoke extracts on the microbial proliferation, including the TMAO reducing microorganisms. No significant differences were observed between TMA-N values of AS and PS samples during storage time.

Peroxide value

Hydroperoxides were determined by peroxide value (PV) analysis. PV values below 5 meq/kg indicate that the fat is fresh or that the hydroperoxides have been degraded into ketones. During marination, PV values increased significantly for the three samples ($P < 0.05$) from 0.35 ± 0.01 meq/kg fish to reach values of 1.50 ± 0.01 , 0.94 ± 0.02 and 0.87 ± 0.04 meq/kg fish for CS, AS and PS samples, respectively (Table 2). During storage (Fig. 1), PVs were significantly greater in the control samples. Protective effect of artichoke leaf and pomegranate peel extracts used on lipid oxidation has been proved. Gokoglu et al. (2009) and Topuz et al. (2014) showed that the use of pomegranate sauce in marinated anchovy reduces lipid oxidation.

No significant difference was observed between AS and PS PV values ($P > 0.05$) during storage despite the significant difference in the DPPH radical scavenging capacity. Piston et al. (2014) showed that DPPH radical scavenging capacity of artichoke depends on solvent used for extraction.

Histamine content

Histamine content can cause adverse health effects for consumers (Kung et al. 2017; Maltar-Strmecki et al. 2013). The fresh fillets showed a concentration of 0.60 ± 0.1 mg/100 g of histamine. Significant differences were found in histamine levels within CS, AS and PS samples after 90 days of storage in olive oil at 4 °C (Fig. 2). The histamine concentration of AS (1.75 mg/100 g) and PS (1.30 mg/100 g) samples were lower than the control sample (2.53 mg/100 g) ($P < 0.05$). Histamine content did not develop beyond the limit (20 mg/100 g) set by the EU (EEC 1991). The lowest histamine content was in PS samples ($P < 0.05$). In fact, the antimicrobial effects of pomegranate peel extracts reduced microbial growth more than artichoke leaf extracts and tend to decrease histamine content.

Free fatty acids

The fatty acid composition of fresh and marinated fillets is presented in Table 3. The predominant fatty acids in fresh fillets are C14:0; C16:0, C18:1 and C22:6n. These results are in agreement with the literature, where C16:0, C18:1

Fig. 2 Histamine contents of fresh and marinated sardine fillets, CS, control sample; AS, samples marinated with artichoke leaf extracts; PS, samples marinated with peel pomegranate extracts

and C22:6n were reported as the most abundant fatty acid in Mediterranean sardines (Ben Rebah et al. 2010; Ennouri et al. 2013; Zlatanov and Laskaridis 2007). Ben Rebah et al. (2010) reported that fatty acid contents of *Sardinella aurita* muscle varies with seasons, it decreased in the summer due to the consumption of fat reserves during the spawning period.

After marination and storage, control samples showed a significant increase in SFA, due to hydrolysis of lipids by lipases, and a significant decrease in PUFA ($P < 0.05$) due

to the oxidation of fatty acids. For samples marinated with artichoke leaf and pomegranate peel extracts, no significant differences were observed in SFA, MUFA and PUFA during storage. This stability is related to the antimicrobial and antioxidant effects of the extracts used. Gokoglu et al. (2009) reported that there is no change in free fatty acid content during the storage of anchovy fillets marinated with pomegranate juice; as for control samples, their free fatty acids values increased until the end of storage.

Sensory analysis

Sensory scores of the three samples are reported in Fig. 3. Appearance, odor, color, tenderness, juiciness, flavor and acid taste were evaluated after 30 days of storage. Darker appearance was observed for PS and AS samples when compared to CS. Penetration of pomegranate peel and artichoke leaf extracts into fish tissue caused the fish to darken. The same result was observed by Gokoglu et al. (2009) and Demirok et al. (2014) in samples of anchovy and rainbow trout marinated with pomegranate sauce, respectively. In this study, panel members preferred a darker color in marinated fish in contrast to the study done by Gokoglu et al. (2009). The odor score was higher ($P < 0.05$) for AS and PS samples compared to control samples. For CS, a negative significant correlation was found between odor scores and TMA ($r = -0.98$, $P < 0.05$) and PV values ($r = -0.99$, $P < 0.05$), respectively. No differences were observed for tenderness, juiciness, flavor and acid taste between the samples

Table 3 Fatty acid composition of fresh and marinated sardine fillets after 90 days of storage

Fatty acid (%)	Fresh fillets	Marinated fillets		
		CS	AS	PS
C14:0	33.2 ± 2.85 ^a	22.2 ± 3.58 ^b	32.1 ± 3.78 ^a	31.9 ± 3.98 ^a
C15:0	0	2.8 ± 0.58	2 ± 0.56	0
C16:0	19.7 ± 1.62 ^a	26.6 ± 3.98 ^b	18.1 ± 2.25 ^a	18.7 ± 1.67 ^a
C17:0	0	2.5 ± 0.89	0	0
C18:0	0	6.1 ± 1.05	2.1 ± 0.78	0
C16:1	0	4.2 ± 0.96	0	3.6 ± 0.89
C18:1 (n-9)	30.8 ± 3.25 ^a	25.3 ± 3.78 ^b	29.4 ± 3.48 ^a	30.1 ± 4.05 ^a
C18:2 (n-6)	4.2 ± 0.52 ^a	3.9 ± 0.09 ^a	3.4 ± 0.99 ^a	4.1 ± 0.05 ^a
C22:6 (n-3)	12.1 ± 1.58 ^a	6.4 ± 0.98 ^b	11.9 ± 1.56 ^a	11.6 ± 1.95 ^a
ΣSAF	52.9	60.2	54.3	50.6
ΣMUFA	30.8	29.5	29.4	33.7
ΣPUFA	16.3	10.3	15.3	15.7

Mean value ± SD (n = 3)

Means in the same line with different superscript letters (a,b) are different ($P < 0.05$)

CS, control samples; AS, samples marinated with artichoke leaf extracts; PS, samples marinated with peel pomegranate extracts

SAF, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid

Fig. 3 Sensory evaluation of marinated sardine fillets, CS, control sample; AS, samples marinated with artichoke leaf extracts; PS, samples marinated with peel pomegranate extracts

($P > 0.05$). PS samples resulted in higher scores for appearance when compared to the other samples ($P < 0.05$).

Conclusion

Pomegranate peel and artichoke leaf aqueous extracts were used to prolong the shelf life of marinated sardine filets. Due to their antimicrobial effects, pomegranate peel and artichoke leaf extracts reduce TVC during marination and storage, they also decrease TVB-N, TMA-N and histamine values. The two extracts showed an inhibitory activity against DPPH which reduces PV values during marination and storage and preserves MUFA and PUFA. Therefore, pomegranate peel extracts may be used as an effective way to improve the quality of marinated sardine fillets. To ameliorate effects of artichoke leaf extract, higher concentration of artichoke leaf powder should be used.

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