Effects of olive oil and olive oil–pomegranate juice sauces on chemical, oxidative and sensorial quality of marinated anchovy

Osman Kadir Topuz *, Pinar Yerlikaya, Ilknur Ucak, Bahar Gumus, Hanife Aydan Büyükbenli

Akdeniz University, Faculty of Fisheries, Department of Seafood Processing Technology, Antalya, Turkey

A R T I C L E I N F O

Article history:
Received 10 October 2013
Received in revised form 18 December 2013
Accepted 29 December 2013
Available online 8 January 2014

Keywords:
Fish marinades
Emulsions
Sauces
Anchovy
Pomegranate juice
Lipid oxidation
Antioxidants

A B S T R A C T

This study describes the potential use of olive oil and olive oil–pomegranate juice sauces as antioxidant, preservative and flavoring agent in fish marinades. The olive oil and sauces, produced from emulsifying of olive oil and pomegranate juice with gums, were blended with marinated anchovy (Engraulis encrasicolus) fillets. The aim of the present study was to produce a new polyphenol-rich marinade sauces by emulsifying pomegranate juice with olive oil in different proportions (25%, 35% and 50% v:v). In order to evaluate the effects of olive oil and olive oil–pomegranate juice sauces on quality of anchovy marinades, the chemical (TVB-N and TMA), oxidative (peroxides value, $K_{230}$, thiobarbituric acid and $K_{270}$) and sensory analyses were carried out during storage at 4 °C. The present study showed that saucing of anchovy marinades with olive oil–pomegranate sauce can retard the undesirable quality changes, prolong the lipid oxidation and improve the sensory properties.

© 2014 Elsevier Ltd. All rights reserved.

1. Introduction

Fish marinades are popular ready-to-eat seafood products in Turkey. Due to its high nutritional value and no necessity of additional preparation by the consumer the consumption of marinades is constantly growing (Szymczak & Kolakowski, 2012). The term "marinades" or "marinated fish" is used to define fish products which consist of fresh, frozen or salted fish or portions of fish processed by treatment with an edible organic acid, usually acetic acid, and salt and put into brines, sauces, or oil (Meyer, 1965). Commercial fish marinades are produced from mainly herring, anchovy (Engraulis encrasicolus) and anchovies because of their high oil content. Anchovy oil contains a high proportion of polyunsaturated fatty acids (PUFA) including eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) known as omega-3 fatty acids which are important fatty acids for the growing up of the retina, brain and nervous system of infants during early life (Wang et al., 2011). NeverthelessPUFAs are susceptible to oxidation which is responsible for rancidity taste and loss in nutritive value. Various synthetic antioxidants, such as butylated hydroxytoluene (BHT) butylated hydroxyanisole (BHA), tert-butyldihydroquinone (TBHQ) and propyl gallate (PG) have been used in various food products in order to prevent lipid oxidation but there is a growing demand for naturally derived antioxidants obtained from wide variety of different plant sources (Kindleysides, Quek, & Miller, 2012).

Generally fish marinades are packed with various vegetable oil or sauces considering to consumers preferences. Although there are many studies about marination of fish, studies on marinated fish packed with sauce are limited. The effects of tomato sauce addition to marinated sardine were studied by Kilinc and Cakli (2005). Gökoglu, Topuz, and Yerlikaya (2009) investigated the effects of pomegranate sauce and sunflower oil on quality of marinated anchovy. The effect of Europe’s most popular cover brine (vinegar, oil and sur cream on the quality of herring meat was investigated by Szymczak, Szymczak, Koronkiewicz, Felisiak, and Bednarek (2013).

Pomegranate (Punica granatum L.) is one of the important fruits grown in Turkey, Iran, USA, Middle East, Mediterranean and Arabic countries. The edible part of the fruit contains considerable amount of acids, sugars, vitamins, polysaccharides, polyphenols and important minerals (Maskan, 2006). Consumption of pomegranate fruit and product such as juice and dressing have enormously increased in recent decade owing to healthful potential of various compounds in pomegranate (Türkylımaz, Tağı, Dereli, & Özkılan, 2013). Pomegranate juice are rich in polyphenols, including ellagittannins, gallotannins, ellagic acids, gallic acids, catechins, anthocyanins, ferulic acids, and quercetins. These polyphenols exhibit various biological activities, such as eliminating free radicals, inhibiting oxidation and microbial growth, and
decreasing the risk of cardio and cerebrovascular diseases and some cancers (Caliskan & Bayazit, 2012).

Olive oil is an important component of the diet of the countries surrounding the Mediterranean Sea. There is also increasing evidence that some of its constituents, mainly phenolic antioxidants, inhibit or modulate oxygen-related reactions and have a substantial favorable effect against oxidative injury. Recently olive oil is gaining interest among consumers of northern Europe, USA, Canada, Australia and other countries, mainly due to the belief that there is a positive role of the Mediterranean diet in the prevention of certain diseases (Boskou & Vissioli, 2003; Paraskevopoulou, Boskou, & Kiosseoglou, 2005). Sauces are very popular oil-in-water emulsion products, which may vary in fat content and viscosity (Dickinson, 1992). Various emulsifiers and stabilisers (e.g., proteins, polysaccharides) are used for obtaining a stable emulsion with a long shelf life in oil-in-water emulsion. Emulsifiers act by one or two mechanisms, including reduction of interfacial tension between oil and water phase, or covering oil droplets with a charged layer to create a physical barrier preventing flocculation (Paraskevopoulou et al., 2005). The effect of concentrated pomegranate juice on quality of marinated anchovy has been investigated in previous work (Gökoğlu et al., 2009). Although pomegranate juice had a good antioxidative effect on quality of marinated anchovy but its effect was limited because of coalescence and stability problems. Thus, the objective of the present study was to develop a “fish marinade sauces” containing olive oil and pomegranate juice that would exhibit reasonable stability over prolonged storage. The effects of sauces on chemical quality, lipid oxidation, sensory properties and shelf life of marinated anchovy were evaluated in this work. The aim of the addition of sauces containing olive oil and pomegranate juice into the marinated anchovy was to protect initial quality, prevent the undesired chemical and oxidative alterations during storage at 4 °C and fulfill the consumer demand.

2. Materials and methods

2.1. Materials

Fresh anchovies (E. encrasicholus) were provided from the fish market of Antalya in January 2013. Anchovies were transferred to laboratory in polystyrene boxes with in an hour after purchase. The fishes were deheaded and eviscerated manually to obtain fillets. Before the marinating, the fish fillets were washed with tap water. Sodium alginate and xanthan gum, purchased from Sigma Chemical Co. (USA) were used as emulsifier. Extra virgin olive oil (Kristal Sızma Izmir-Turkey) and ripened pomegranate fruits (punica granatum L.) were purchased from a local market in Antalya-Turkey. The pomegranate peel, pulp and arils were separated manually. Then the pomegranate juice was extracted from fresh arils with using mechanical press. The brix of pomegranate juice, measured with hand refractometer (WYT-4, Quanzhou, China) was 16.1 °Bx.

2.2. Preparation of olive oil–pomegranate juice sauce

Three olive oil–pomegranate juice emulsions containing 25%, 35% and 50% (v/v) pomegranate juice were prepared according to method of Paraskevopoulou, Boskou, and Paraskevopoulou (2007): A pomegranate juice polysaccharide solution was first prepared by slowly dispersing emulsifiers (0.5% w/v sodium alginate and 0.5% w/v xanthan gum) with stirring for at least 4 h, to ensure complete dissolution. The emulsions were prepared by adding dropwise of virgin olive oil to the pomegranate juice-polysaccharide solution, while mixing with a propeller-type mechanical stirrer. 50%, 35%, 25% and 0% (v/v) pomegranate juice polysaccharide solution containing sauces were denoted as S50, S35, S25 and S0 respectively. All emulsified sauces were stirred with a vortex for 2 min. The droplet size of the resulting crude emulsion was then reduced further using an Ultra-Turrax T25 homogeniser (IKA Instruments, Germany). After the emulsions had been properly formed, olive oil and olive oil–pomegranate juice emulsions were stored in screw-capped glass containers at cold temperature (4 ± 1 °C) prior to use as marinating sauce.

2.3. Marination process

Marination process was carried out according to the method of Gökoğlu et al. (2009) with a slight modification. All anchovy fillets were immersed into a marinating solution consisting of 2% (v:v) acetic acid and 10% (w:v) NaCl salt, with a final pH of 2.35. The fish to solution ratio was 1:1.5 (w:v), and the marination mixture was stirred at 3 h intervals. The marinating process, performed at ambient temperature (20 ± 1 °C) was completed within 30 h. After marination process, the fish fillets were removed from the marinating solution and left to drain on sterile stainless steel wire mesh for 5 min. Before adding olive oil–pomegranate sauce to the marinated anchovy, preliminary trials were performed in order to determine the suitable concentration in terms of taste. Thus the selected ratio of olive oil–pomegranate juice to marinated anchovy filet was 1:4 (v:v). Marinated anchovy fillets were divided into five groups. First group was packaged without sauce in polyethylene bags (HDPE bags), and denoted as MC. Other groups were packaged with sauces containing different concentrations pomegranate juice. The samples treated with S50, S35, S25 and S0 sauces were denoted as M50, M35, M25 and M0. Before the analysis sauces and oil was removed from anchovy fillets with using laboratory paper towel. All samples were stored at cold temperature (4 ± 1 °C) for 100 days and analysed to determine the quality changes at 20 days intervals. Once the sauces were added to marinated anchovy fillets, the initial analyses were performed in same day (day 0).

2.4. Analyses

2.4.1. Total phenolic content

The total aqueous and lipid soluble phenolic content were determined separately using the Folin–Ciocalteu reagent with gallic acid as a standard in the range 0–1.0 mg/ml (Thomas, Bernards, Drake, & Guglielmo, 2010). The aqueous (phenolics soluble in aqueous solution) and lipid soluble (phenolics soluble in organic solvent such as acetone) phenolic contents were determined by adding 500 μl of the samples extracted with 50 mM sodium phosphate buffer (pH 7.5) and acetone, respectively, to 1 ml of ultra pure water and 2.5 ml Folin–Ciocalteu reagent (diluted 10-fold) in test tubes. The test tubes with the mixture were incubated in the dark at room temperature for 1 h. After incubation, 200 μl of each sample mixture were placed in spectrometer (Thermo Scientific Evolution 160 UV–VIS, Germany) wells and the absorbance measured at 755 nm. The results were expressed as mg gallic acid equivalents/ml sample (GAE/ml sample). Values for total phenols were determined by summation of the aqueous and lipid soluble phenol values.

2.4.2. Antioxidant activity

The hydrophilic and lipophilic antioxidant activities of olive oil (S0), pomegranate juice (P) and olive oil–pomegranate sauces (S25, S35 and S50) were measured according to the method of Thomas et al. (2010) with slight modification. This method is based on the capacity of a sample to scavenge the ABTS radical caption compared to standard antioxidant (Trolox) in a dose–response curve (0–20 μM).
For the hydrophilic antioxidant activity (HAA), the reaction mixture consisted of 2 mM ABTS, 30 μM H₂O₂ and 0.25 μM horse reddish peroxidase (HRP) in 50 mM sodium phosphate buffer (pH 7.5) in a total volume of 1 ml. The reaction mixture was diluted 1:5 with sodium phosphate buffer and monitored using the kinetics mode at 730 nm until stable absorbance was observed. The assay temperature was 25 °C. Then 10 ml of the sample extracted with sodium phosphate buffer was added to 250 ml of the reaction mixture and the decrease in absorbance, which is proportional to the ABTS’ quenched, was determined after 5 min using spectrometer (Thermo Scientific Evolution 160 UV–VIS, Germany).

For the lipophilic antioxidant activity (LAA), the reaction mixture contained 1 mM ABTS, 30 μM H₂O₂ and 6 mM HRP in acified ethanol (phosphoric acid 0.7, v/v) in a total volume of 1 ml. The reaction mixture was diluted 1:3 with acified ethanol and monitored using the kinetics mode at 730 nm until a stable absorbance was observed. Then 10 μl of the sample extracted with HPLC grade acetone was added to 250 μl of the reaction mixture, and the decrease in absorbance at 730 nm was determined after 5 min. In both the hydrophilic and lipophilic antioxidant analysis, the decrease in absorbance was determined from the difference between the absorbance values at 730 nm before and after sample addition. Antioxidant activities were expressed as μmol Trolox equivalents/ml sample. Total antioxidant activity (TAA) was determined by summing the HAA and LAA values.

2.4.3. pH

The pH value was determined by dipping a pH electrode into homogenates of filleted anchovy muscle in distilled water (1:1). All measurements were performed at room temperature (24 ± 1 °C) using pH-meter (Thermo Scientific Orion 2-star, Germany).

2.4.4. Total volatile basic nitrogen (TVB-N)

A 10 g homogenised fish filet sample was washed into the distillation flask and 1 mg magnesium oxide (Merck, Darmstadt, Germany) was added with a drop or two of silicone anti-foam solution (Fluka, Steinheim, Germany). Samples were boiled and distiled into a 50 ml conical flask with added tashiro-indicator (Riedelde Haen, Seelze, Germany). After distillation, the contents of conical flask were titrated with 0.1 mol equi/L NaOH (Merck, Darmstadt, Germany) (Schormüller, 1968).

2.4.5. Trimethylamine (TMA)

A 10 g homogenised fish sample was blended with 90 ml of 5 g/100 ml trichloroacetic acid (TCA) (Merck, Darmstadt, Germany) using an ultraturrax homogeniser and filtered. A 4 ml aliquot was transferred into test tubes and 1 ml formaldehyde (20 ml/100 ml) (Merck, Darmstadt, Germany), 10 ml anhydrous toluene (Merck, Darmstadt, Germany), 3 ml KOH (50 g/100 ml) (Merck, Darmstadt, Germany) solution were added. The tubes were shaken and a 5 ml toluene layer was pipetted into the flask containing 5 ml picric acid (0.02 g/100 ml) (Merck, Darmstadt, Germany). The supernatant was then transferred to a spectrophotometric cell. The absorbance of the mixture at 410 nm was measured. At the same time a series of standards were prepared and measured (Schormüller, 1968).

2.4.6. Oxidative stability evaluation

Peroxide value (PV) and specific extinction (K_{232} mg) assays, as an index for primary lipid oxidation, were carried out according to analytical methods described in Regulation EC/2568/91 of the Commission of the European Union (EU, 1991). Peroxide value, expressed as ml equivalents of active oxygen per kg of fish oil (meq/kg), was determined as follows: a mixture of oil and chloroform/acetic acid was left to react with a solution of potassium iodide in darkness; the free iodine was then titrated with a sodium thiosulfate solution.

The 2-thiobarbituric acid (TBA) and specific extinction (K_{270} mg) assays, as an index for secondary lipid oxidation, were carried out according to the procedure of Schmedes and Holmer (1989) and EU (1991), respectively. TBA value, expressed as mg malonaldehyde (MA) per kg of fish sample, was determined as follows: homogenised fish sample (10 g) was mixed with 25 ml of 20% trichloroacetic acid (w/v) and homogenised in a blender for 30 s. After filtration, 2 ml of the filtrate were added to 2 ml of 0.02 M aqueous TBA in a test tube. The test tubes were incubated at room temperature in the dark for 20 h; then the absorbance was measured at 532 nm by using a UV–VIS spectrophotometer (Thermo Scientific Evolution 160 UV–VIS, Germany).

K_{232} and K_{270} extinction coefficients were calculated from the adsorption of a solution of the fish oil, extracted from anchovy fish fillet samples, in iso-octane at 232 and 270 nm, using a UV spectrophotometer (Thermo Scientific Evolution 160 UV–VIS, Germany).

2.4.7. Sensory analysis

Sensory analysis of anchovy marinades was performed by a panel of ten panelists. The panelists were from the staff of Food Engineering Department who had experience in evaluating seafood. The evaluations were performed in separated sensory test boxes under normal daylight and ambient temperature. Five marinated anchovy fillets were served in white porcelain trays. The panelist used water and piece of bread to clean their palate between samples. The panelists were not informed about the experimental approach and the samples were blind-coded with 3-digit random numbers. The panelists evaluated the samples for odour, appearance and taste and overall acceptance on a nine-point hedonic scale (Amerina, Pangborn, & Roessler, 1965). A score of 9–7 indicated “very good”, a score of 6.9–4.0 “good”, a score of 3.9–1.0 denoted as spoiled.

2.4.8. Statistical analysis

All measurements were carried out in duplicate. Data were subjected to analysis of variance (ANOVA) using the General Linear Models procedure of the Statistical Analysis System software of SAS Institute. Differences among the mean values of the various treatments and storage periods were determined by the least significant difference (LSD) test, and the significance was defined at p < 0.05.

3. Result and discussion

3.1. Total phenolics content

Phenolic compounds of plant origin have attracted considerable attention due to their beneficial functional and nutritional effects including antioxidant and antimicrobial activity. In addition to extending shelf-life of foods by inhibition of lipid peroxidation, the phenolics act in the scavenging of free radical and can protect the human body against damage caused by them (Bubonja-Sonje, Giacometti, & Abram, 2011). Olive oil and pomegranate juice are an excellent source of natural antioxidants. Total phenolic concentrations in pomegranate juice varies within the limits of 0.2–1.0%, depending on variety and include mainly tannins, ellagic tannins, anthocyanins, catechins, gallic and ellagic acids (Ignarro, Byrns, Sumi, de Nigris, & Napoli, 2006). In this study the aqueous total phenolic contents of pomegranate juice (PJ), olive oil (S0) and olive oil–pomegranate sauces (S_{25}, S_{50}, and S_{75}) ranged from 3.36 to 127.34 mg GAE/100 ml while the lipid soluble phenolic content of PJ, S_{0}, S_{25}, S_{50} and S_{75} ranged from 2.31 to 45.53 mg GAE/100 ml (Table 1). The lowest total phenolic content was...
Table 1

<table>
<thead>
<tr>
<th>Samples**</th>
<th>Total phenolic content (mg gallic acid equivalent/100 ml sample)</th>
<th>Antioxidant activities (μmol trolox equivalent/ml sample)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Aqueous Lipid soluble Total</td>
<td>Hydrophilic Lipophilic Total</td>
</tr>
<tr>
<td>PJ</td>
<td>127.34 ± 1.36&lt;sup&gt;a&lt;/sup&gt; 2.31 ± 0.27&lt;sup&gt;c&lt;/sup&gt; 132.72 ± 3.21&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.35 ± 0.08&lt;sup&gt;a&lt;/sup&gt; 0.01 ± 0.01&lt;sup&gt;c&lt;/sup&gt; 2.36 ± 0.04&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>S&lt;sub&gt;25&lt;/sub&gt;</td>
<td>3.36 ± 0.85&lt;sup&gt;a&lt;/sup&gt; 45.53 ± 1.09&lt;sup&gt;b&lt;/sup&gt; 52.89 ± 1.54&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.04 ± 0.01&lt;sup&gt;b&lt;/sup&gt; 0.25 ± 0.01&lt;sup&gt;b&lt;/sup&gt; 0.29 ± 0.02&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>S&lt;sub&gt;25&lt;/sub&gt;</td>
<td>38.19 ± 0.82&lt;sup&gt;d&lt;/sup&gt; 16.28 ± 0.86&lt;sup&gt;b&lt;/sup&gt; 54.47 ± 1.19&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.49 ± 0.02&lt;sup&gt;d&lt;/sup&gt; 0.47 ± 0.02&lt;sup&gt;d&lt;/sup&gt; 0.96 ± 0.03&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>S&lt;sub&gt;25&lt;/sub&gt;</td>
<td>47.35 ± 1.88&lt;sup&gt;c&lt;/sup&gt; 14.56 ± 0.72&lt;sup&gt;c&lt;/sup&gt; 61.91 ± 1.44&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.72 ± 0.03&lt;sup&gt;c&lt;/sup&gt; 0.45 ± 0.03&lt;sup&gt;c&lt;/sup&gt; 1.17 ± 0.04&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>S&lt;sub&gt;50&lt;/sub&gt;</td>
<td>64.81 ± 1.24&lt;sup&gt;b&lt;/sup&gt; 13.53 ± 0.81&lt;sup&gt;d&lt;/sup&gt; 78.34 ± 1.15&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.89 ± 0.03&lt;sup&gt;d&lt;/sup&gt; 0.43 ± 0.02&lt;sup&gt;d&lt;/sup&gt; 1.32 ± 0.05&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

* The values represent ± standard errors; n = 3 per experimental replicate; means within the same column (a, b, c, d, e) with different letters are different (p < 0.05).
** PJ, pomegranate juice; S<sub>0</sub>, olive oil; S<sub>25</sub>, 25% pomegranate juice containing sauce; S<sub>50</sub>, 35% pomegranate juice containing sauce; S<sub>50</sub>, 50% pomegranate juice containing sauce.

3.2. Antioxidant activity

In order to obtain an accurate value for the total antioxidant activity, both the hydrophilic and lipophilic antioxidant activity analyses were done in the same samples and the total antioxidant activities of samples were calculated by summation of the hydrophilic and lipophilic antioxidant activity values. The hydrophilic, lipophilic and total antioxidant activity values of PJ, S<sub>0</sub>, S<sub>25</sub>, S<sub>35</sub> and S<sub>50</sub> varied widely and ranged from 0.01 to 2.36 μmol trolox equivalent/ml (Table 1). It was observed that the addition of PJ to sauces significantly (p < 0.05) changed the hydrophilic, lipophilic and total antioxidant activity values of sauces. The hydrophilic antioxidant activity made up more than 70% of the total antioxidant activity in the sauces and pomegranate juice addition, while a positive correlation (r: 0.993) has been observed between lipophilic antioxidant activity and pomegranate juice addition while a positive correlation (r: 0.997) has been observed between hydrophilic antioxidant activity and pomegranate juice addition with a positive correlation (r: 0.979) has been observed between hydrophilic antioxidant activity and pomegranate juice addition to S<sub>25</sub>, S<sub>35</sub> and S<sub>50</sub> sauces. The lowest (0.29 μmol trolox equivalent/ml) total antioxidant activity was determined in olive oil, while the highest (2.36 μmol trolox equivalent/ml) was determined in PJ. The total antioxidant activity value of S<sub>0</sub> (0.29) was similar with those (0.27 μmol trolox equivalent/ml) reported by Bubonja-Sonje et al. (2011). The total antioxidant activity value of PJ (2.36) was significantly (p < 0.05) lower than those (2.55) reported by Gökoglu et al. (2009) and those (from 32.16 to 46.42 μmol trolox equivalent/ml) reported by Türkyılmaz et al. (2013).

3.3. pH value

The initial pH of the raw anchovy fillets was 6.49. During the marinating of anchovy fillets, a considerable decrease in the pH determined in S<sub>0</sub> (52.89), while the highest was determined in PJ (132.72). Aqueous phenolic content of all sauces was higher than its lipid soluble phenolic content. A negative correlation (r: −0.931) has been found between aqueous and lipid soluble phenolic content in S<sub>25</sub>, S<sub>35</sub> and S<sub>50</sub> sauces. The addition of pomegranate juice to olive oil significantly (p < 0.05) affected the both aqueous and lipid soluble phenolic content of sauces and the lowest total phenolic content was determined in the sauce containing lowest pomegranate juice. The total phenolic contents of PJ (132.75 mg GAЕ/100 g), S<sub>0</sub> (78.34 mg GAЕ/100 g) and S<sub>35</sub> (61.91 mg GAЕ/100 g) obtained in this study were higher than those (56 mg GAЕ/100 g) reported by Gökoglu, Topuz, and Yerlikaya (2009) and lower than those (180–210 mg GAЕ/100 ml) reported by Türkyılmaz et al. (2013). The total phenolic content of olive oil determined in this study was almost two fold higher than those (27.9 mg GAЕ/100 g) reported by Bubonja-Sonje et al. (2011). The differences between different results could be explained by different extraction conditions (solvent, temperature and extraction time), pomegranate and olive variety and growing conditions.

3.4. Total volatile bases nitrogen (TVB-N)

TVB-N analysis is more useful in assessing the degree of fish spoilage than in evaluating the changes occurring during the first stages of storage. TVB-N is a general term which includes the measurement of trimethylamine (TMA), dimethylamine (DMA), ammonia, and other volatile basic nitrogenous compounds associated with seafood spoilage (Huss, 1995; Sallam et al., 2007). The initial TVB-N in raw anchovy was 9.82 mg/100 g fish filet. A decrease was observed in TVB-N values, from 9.82 to 8.97, after the marinating process of anchovy fillets. During the storage, gradually and increases in TVB-N values were observed by the 40 days, for all samples, after 40 days of storage significantly (p < 0.05) increases in TVB-N values were observed for all marinated samples (Table 2). Higher TVB-N values were found in MC compared to those M0, M25, M35 and M50-treated anchovy samples. Although the control sample (MC) reached the maximum acceptable freshness limit of 35 mg TVB-N/100 g fish fillet (Ludorf & Meyer, 1973) at the end of the storage, all sauces samples (M0, M25, M35 and M50) were still below this limit. It is indicating that the sauces have significantly effect on reduction of TVB-N content in marinated fish. A similar pattern of the increase in TVB-N was reported for Pacific saury (Sallam et al., 2007), for marinated anchovy sauced with concentrated...
pomegranate juice (Gökoglu et al., 2009) and for sardine marinades sauced with tomato during refrigerated storage. On the other hand, TVB-N content of tomato sauced sardine marinades stored for 6 months at 4°C was found a level of 8.21 mg TVB-N/100 g (Kilinc & Cakli, 2005).

3.5. Trimethylamine (TMA)

The TMA, produced from the reduction of trimethylamine oxide (TMAO) by bacterial activity has been used to determine fish quality. It has been suggested that a maximum TMA value, indicating the good quality of the fresh fish, is 1 mg/100 g, while fish may be consumed up to a TMA value of 10 mg/100 g (FAO, 1986; Kilinc & Cakli, 2005). In this study, the TMA value of fresh anchovy was 0.82 mg/100 g. After the marination process, an initial TMA value from 0.82 to 0.91 mg/100 g was measured for marinated anchovy. TMA values of all samples, particularly MC sample significantly increased (p < 0.05) during the storage (Table 2). By the end of the storage period, MC presented a high level of 7.34, while all sauced samples (M0, M25, M35 and M50) showed significantly lower TMA values of 3.41, 4.19, 5.27 and 5.84 mg/100 g, respectively. TMA levels in all marinades of the present study were significantly lower TMA values from 0.82 to 0.91 mg/100 g was measured for marinated anchovy samples than the samples sauced with M0 may be resulted from the inhibitory effect of the pomegranate juice on the microbial proliferation, including the TMAO reducing microorganisms.

3.6. Changes in lipid oxidation products

While primary oxidation products such as hydroperoxides and conjugated dienes were determined by using peroxide value (PV) and K232 extinction coefficient analyses, secondary oxidation products such as thiobarbituric acid (TBA), carbonyls, aldehydes and conjugated triens were determined by using TBA and K270 extinction coefficient analyses.

3.6.1. Peroxide value (PV)

Hydroperoxides, known as primary oxidation products, were determined by peroxide value (PV) analysis. The peroxides values of anchovy marinades sauced with both olive oil and olive oil–pomegranate sauces are shown in Fig. 1. The gradually increases in PV were determined in all samples during the first 40 days.
While the PV of MC and M0 samples significantly increased, PV of M25-, M35- and M50-treated anchovy samples gradually increased up to 100 days of storage. When PV of all samples were compared, it was determined that PV of the MC sample was significantly (p < 0.05) higher than the other sauced samples contained different concentrations of olive oil–pomegranate juice sauce and olive oil throughout the storage, particularly after 40 days.

The result indicated that samples treated with sauce S25 and S35 demonstrated a similar effect on inhibiting the production of peroxides in anchovy marinades samples but their inhibiting effect was significantly (p < 0.05) lower than that of S50. After 100 days of storage, the M0 sample reached 15.56 meq/kg fish fillet, showing an antioxidative effect compared with the sample M50 demonstrated the best antioxidant effect (7.12 meq/kg meat) on inhibiting of hydroperoxide production in anchovy fish marinades.

### 3.6.2. K232 extinction coefficients

The measurement of the K232 specific extinction coefficient was used to determine the level of conjugated dienes present in the emulsified sauces. It is known that the oxidation products of oils and fats, which may result from their decomposition, display characteristic spectra in the ultraviolet region and at about 232 nm. Therefore, a determination of the absorbance at 232 nm is an indication of the state of oxidation of a oils (Paraskevopoulou et al., 2005). In this study the K232 coefficient demonstrated a significantly (p < 0.05) higher initial values for MC and M0 sample in comparison to M25-, M35- and M50-treated anchovy samples. The K232 coefficient of all samples significantly (p < 0.05) increased during the first 60 days of storage (Fig. 1). K232 coefficients of M25-, M35- and M50-treated anchovy samples remained constant after the 60 days of storage in comparison to MC and M0 samples indicating an inhibition in the formation of primary oxidation products. This is probably due to high amount of phenolic compounds present in pomegranate juice or maybe because of the delayed development of hydroperoxides in the emulsions. The lowest K232 coefficient was determined for the M50 sample (2.62), while the highest was determined for MC sample (2.96) at the end of the storage. Higher K232 coefficients were found for M0 sample than those samples sauced with M25, M35 and M50 during the storage, indicating that all pomegranate–olive oil sauces are more effective than olive oil on the inhibition of lipid oxidation in marinades.

### 3.6.3. Thiobarbituric acid (TBA) values

Thiobarbituric acid is used to determine secondary oxidation products in oil and oily foods (Shahidi & Wanasundara, 1998). The TBA value of fish oil extracted from fresh anchovy was 2.02 malondialdehyde/kg (mg MA/kg), after the marinating process, the TBA value increased from 2.02 to 2.36 mg MA/kg. While initial TBA values of samples ranged between 0.82 and 1.36 mg MA/kg, TBA values in all samples increased during the first 40 days of storage (Fig. 2). There was a significant increase in TBA values of all samples after 40 days of storage (p < 0.05). TBA value is the indicator of secondary oxidation products. After the 40 days of storage, the TBA values of MC and M0 samples significantly increased from 1.63 to 2.31 and from 1.27 to 1.84 mg MA/kg, respectively. The lowest TBA value (1.36 mg MA/kg fish meat) was determined for M50 sample at the end of the storage (Fig. 2). The fish samples sauced with low concentrations of pomegranate juice (M25 and M35) showed similar behaviour during the storage. The antioxidant activities of S25 and S35 sauces were weaker than those of S50 sauce. The sauce S50, which containing high proportion of pomegranate juice, showed the highest antioxidant activity, followed by S35 and S25, while S0 showed the lowest antioxidant activity during the storage of samples. The degradation of hydroperoxides, producing secondary oxidation products caused an increase in the level of secondary oxidation products (Maqsood & Benjakul, 2010). Constantly increase in the TBA values of all samples may indicate the formation of primary lipid oxidation products to secondary lipid oxidation products during the 100 days of storage.

### 3.6.4. K270 extinction coefficients

The K232 specific extinction coefficient indicates the intermediate compounds of the peroxidation catalysed by the lipoxygenase, indicating the amount of hydroperoxides, while the K270 specific extinction coefficient indicate the amount of secondary oxidation products (Weber, Bochi, Ribeiro, Victorio, & Emanuelli, 2008). K270 coefficient is an important quality index, indicating formation of secondary lipid oxidation products such as conjugated triens. In this study, the K270 coefficient of fresh anchovy fillet was 0.14. After the marinating processes, an increase in K270 coefficient from 0.14 to 0.16 was measured for marinated anchovy fillets (Fig. 2). The addition of all sauces did not produce a significant reduction in the K270 coefficients and only a minor decrease was recognised in the initial K270 coefficients after the addition of all sauces. The K270 coefficients of all the samples increased during the first 60 days; increase of K270 coefficients of all the samples accelerated after 60 days. A significant difference (p < 0.05) in K270 coefficients was observed between the control and sauced samples throughout the storage.

The delays in lipid oxidation induced by the three different olive oil–pomegranate juices (S125, S35 and S50) were better than those of olive oil sauce (S0). At the end of the storage, the K270 coefficients of M25-, M35- and M50-treated anchovy samples were below 0.22, whereas the MC and M0 samples reached 0.27 and 0.24 K270 coefficient values, respectively, indicating the significant
(p < 0.05) antioxidant effect of S25, S35 and S50 sauces. The sauce S50, containing 50% pomegranate juice, showed the best antioxidant effect on lipid oxidation of marinated anchovy fillet and yielded the lowest (0.16) $k_{270}$ coefficient in M50 samples. The present study reveals that the type of sauces and concentrations of pomegranate juice in sauces are a key condition for marinated fish fillet storage at refrigerated temperature.

### 3.7. Sensory attributes

Sensory evaluation is the most popular way of assessing the freshness of fish. It is fast, simple, and provides immediate quality information. The sensory characteristics of fish are clearly visible to the consumer and are essential for consumer satisfaction (Reineccius, 1990; Sallam et al., 2007). At the end of the storage, panelist considered that the control and olive oil sauced samples to be unfit for human consumption as the samples were soft with bitter taste and lack of the typical fish marinades odour and presence of ammonia off-odour. This was also indicated by the chemical and oxidative analyses measurements during the storage.

### 4. Conclusion

It can be concluded that the olive oil–pomegranate juice sauces (S25, S35 and S50) have the ability to delay chemical changes, inhibit lipid oxidation, maintain the sensory attributes and extend the shelf life in addition to their desirable taste and flavour; therefore, considering the consumer preference for natural additives such as natural preservative and antioxidants, olive oil–pomegranate sauces can be used as a natural antioxidant, preservative and flavoring additive in fish marinades such as anchovy marinades. Besides, antioxidant and preservative effect of sauces increased parallel with increasing of pomegranate juice concentration in sauces. On the other hand, using of virgin olive oil presented also better organoleptic sauce properties providing an attractive yellow colour, as well as an acceptable content of bioactive compounds and moderate antioxidant activity. Although olive oil–pomegranate sauces contributed to product quality and sensory properties, the dark colour and stability of emulsified marinades sauces over prolonged storage should be investigated in further studies.

### Acknowledgement

The Scientific Research Project Administration Unit of Akdeniz University supported this research.

### References


