



INCREASING OXIDATIVE STABILITY OF PRECOOKED TROUT FILLET USING HERBAL ESSENTIAL OILS

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Summary

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The antioxidant effect of tarragon and summer savory herbal essential oils (EOs) on precooked trout fillets during frozen storage period was investigated in this study. Three groups of fish fillets were treated with tarragon EO and three other groups were treated with summer savory EO and then cooked by different cooking methods (frying, oven baking and steaming). During the storage period, fat hydrolysis was evaluated through measuring free fatty acid value (FFA) and oxidation products were measured via peroxide value (PV) and thiobarbituric acid reactive substances value (TBARS). During the storage period, the amount of FFA was high in oven baked samples (0.34–0.53% oleic acid) and steamed fillets (0.56–0.84% oleic acid). Following the control, the highest PV was obtained from fried fillets treated with summer savory (4.53–4.67 meq/kg) ($P < 0.05$). Also, TBARS in fried and steamed samples containing summer savory was higher than samples containing tarragon ($P < 0.05$). Overall acceptability score of antioxidant treated samples was higher than that of controls ($P < 0.05$). The results of this study showed that tarragon and summer savory essential oils retarded the oxidation during frozen storage and samples treated with tarragon showed slower hydroperoxide and malonaldehyde formation than those of summer savory-treated or the control samples.

Key words: antioxidant activity, storage, summer savory, tarragon, trout

INTRODUCTION

Fish meat, compared to the other meat products, is more vulnerable to oxidative spoilage, deterioration of colour, odour, flavour, texture, and the production of toxic compounds, since it contains a high level of unsaturated fatty acids (Kanner, 1994). It is reported that cutting, mincing, and cooking affect the lipid stability of the

product due to increasing product surface area and expose phospholipid fractions and intramuscular fat to oxidising agents, introduce interstitial air and accelerate oxidative reactions (Mc Bride *et al.*, 2007).

In order to retard or prevent lipid oxidation, maintain the good quality and ex-

tends the shelf-life of food products, antioxidants are applied. Due to increasing consumers and manufacturers' awareness of the possible toxicity of synthetic preservatives, interest in natural antioxidants has increased in recent decades, both on industry and consumer side (Vareltzis *et al.*, 1997). Several studies in relation to the potential of herbs and spices as natural antioxidants have been reported (Tsai *et al.*, 2005; Azizkhani & Tooryan, 2015).

Among the herbs reported to have antioxidant components, tarragon and summer savory are widely used in many food products in Iran. Tarragon (*Artemisia dracunculus* L.), also known as estragon, is a species of perennial herb in the sunflower family which is cultivated for culinary and medicinal purposes. Essential oil (EO) of tarragon contains considerable amount of phenylpropanoids such as methyl eugenol and methyl chavicol. Also, analysis of tarragon EO revealed the presence of trans-anethole, alpha-transocimene, limonene, alpha-pinene, and bornyl acetate as the main components (Ayoughi *et al.*, 2011).

Satureja hotensis L. (summer savory) is an aromatic plant of the family Lamiaceae. The antioxidant activity of summer savory is due to the phenolic compounds, carvacrol and thymol and also the major non-phenolic para-cymene, alpha and gamma-terpinene (Gulluce *et al.*, 2003; Adiguzel *et al.*, 2007).

As heat processing and subsequent storage of the cooked fish products containing considerable amounts of unsaturated fatty acids increase the formation of oxidised off-flavours (Mc Bride *et al.*, 2007), food industry has an interest in new approaches that allow fish products to be processed with less oxidative deterioration. The objective of this study was to evaluate lipid stability of rainbow trout

fillets treated with tarragon and summer savory EOs and subjected to three cooking methods (frying, oven baking and steaming) during a 4-month frozen storage period.

MATERIALS AND METHODS

Essential oils

EOs of *Artemisia dracunculus* L. and *Satureja hotensis* L. were obtained from Barij company, Kashan, Iran. The EOs were stored in airtight dark glass vials at 4°C.

Gas chromatography/Mass spectroscopy of essential oils

EOs were analysed by gas chromatography (GC; Thermo Quest 2000, Finnigan, UK). The chromatograph was equipped with a DB5 capillary column (30 m × 0.25 mm ID × 0.25 mm film thickness) and the data were acquired under the following conditions: initial temperature 50 °C; program rate 2.5 °C, final temperature 265 °C; injector temperature 250 °C and injection volume 1 µL. The carrier gas was helium, flow rate 2 mL/min and the split ratio was 120. The EOs were also analysed by GC mass spectroscopy (MS) (MS detector model Thermo AS2000, Thermo Quest) using the same capillary column and analytical conditions indicated earlier. The MS was run in the electron ionisation mode, using ionisation energy of 70 eV. Identification of the components was based on the comparison of their relative retention time and mass spectra with those of standards (Adams, 2001). The peaks were tentatively identified based on library search using NIST and Wiley Registry 8 Edition. As reference points in the calculation of relative retention indices, alkanes were used.

Sample preparation

Thirty samples of rainbow trout (*Oncorhynchus mykiss*), between 500 and 700 g in weight and average length of 380 mm, were purchased from the local wet market, Mahmoudabad, Iran, in April 2017. Having been transferred on ice to the laboratory within 2 h, the fish were beheaded, gutted and washed. Then, they were filleted (totally 180 fillets). Three groups of fillets (45 fillets) were treated with tarragon EO, three groups (45 fillets) were treated with summer savory EO and three other groups (45 fillets) were treated with butylated hydroxytoluene (BHT) (Sigma Aldrich, Germany). Also, three groups (45 fillets) were used as control. For each cooking treatment, 4 groups were considered (control, treated with tarragon EO, summer savory EO or BHT). The EOs and BHT were applied to the fish as described by Akhtar *et al.* (1998). Fillets were immediately immersed in BHT (2 mg/mL ethanol 70%) and EOs (20 mL/L) in distilled water for 2 min and cooked by different cooking methods. Sample fillets were deep-fried for about 4 min in sunflower oil at 180 °C, oven baked for 22 min at 200 °C and steamed for 10 min at 200 °C. Three groups without antioxidant were also cooked by the same methods. One of each precooked samples (with and without antioxidant) were analysed immediately, and the rest of samples were stored in a deep freezer (-18 °C) in refrigerator bags for 4 months, samples were analysed monthly.

Lipid extraction

The extraction of lipid was conducted according to the method described by Mortensen *et al.* (2002). About 10 g of each sample was transferred to a 500 mL plastic centrifuge tube and 200 mL of chloroform:methanol (v/v ratio 7:3) was

added. The mixture was homogenised using a homogeniser (Heidolph, Diox 600, Kelheim, Germany), 50 mL of 1 mM CaCl₂ was added to the suspension and shaken for 10 s. The mixture was then centrifuged for 30 min at 20 °C at 1,400×g, the supernatant was transferred to a 500 mL separation funnel to collect the chloroform (lower) layer. The upper layer and the solids were combined and mixed with 150 mL of chloroform. The mixture was homogenised and then centrifuged as mentioned above. This procedure was repeated once more. The three separated chloroform layers were combined and poured into a 1,000 mL conical flask, and the chloroform was evaporated at 60 °C using a Buchi vacuum Rotavapor R-215 (Büchi Labortechnik AG, Flawil, Switzerland). The remaining oil was then flushed with nitrogen and used for future analysis.

Free fatty acids (FFA)

FFA contents, expressed as percentage of oleic acid, was determined by the acidimetric titration of the Bligh and Dyer extracts after adding ethanol and using phenolphthalein as an indicator, following AOCS (1994).

Peroxide value (PV)

Lipid samples (0.02 g) were weighed into a 250 mL volumetric flask and 15 mL of chloroform:methanol (v/v 7:3) was added. To each sample were added 0.2 mL 1% ferrous chloride and 0.2 mL of 4 M ammonium thiocyanate, and the final volume was made up to 25 mL using chloroform:methanol (v/v 7:3). Samples were mixed and kept under dimmed light for 5 min for absorbance determination at 505 nm. The result was expressed as milliequivalents of oxygen per kilogram of lipid. A sample blank and a reagent blank

were also used. All the measurements were carried out in triplicate. A standard curve was determined under the same condition using ammonium ferric sulfate (AR grade) as standard (Ye *et al.*, 2009).

Thiobarbituric acid reactive substances

Thiobarbituric acid reactive substances (TBARS) were measured using a method described by Kristensen *et al.* (2001). The thiobarbituric acid (TBA) reagent was prepared by mixing equal volumes of freshly prepared 0.025 M TBA and 2 M H₃PO₄/2 M citric acid. The combination of citric acid and phosphoric acid was used as both acidulants and metal chelators. To 6.0 g of sample, exactly weighed, was added 18.00 mL of TBA reagent, and the resulting mixture was homogenised using an Ultra Turrax (Heidolph, Dixa 600, Germany) for 2 min until the mixture appeared to be homogeneous. Then, 6 mL of the suspension was transferred to a Pyrex tube to which 3.5 mL of chloroform was added, followed by gentle mixing for 5 min. The mixture was centrifuged at 6000×g for 15 min at room temperature. The aqueous layer was transferred to another test tube, which was placed in a water bath at 100 °C for 10 min, followed by cooling on ice. The orange-red cyclohexanone supernatant was decanted and its absorbance at 532 nm was measured spectrophotometrically (BSA 3000 Chemistry Analyzer, SFRI, Saint Jean d'Ilac, France). The results were expressed as milligrams of malonaldehyde (MA) per kilogram of fish flesh as following formula:

$$\text{MA (mg/kg)} = (\text{Absorbance}_{\text{sample}} \times \text{Volume}_{\text{extraction}} \times \text{Volume}_{\text{dilution}}) / (\text{slope} \times \text{weight of sample})$$

The slope (0.21) was calculated by plotting standard curve with 1,1,3,3-tetra-

ethoxypropane (TEP). The results were recorded as μmol TEP/mL equivalent to μmol MA/mL. As a result, 10 μmol MA/10 mL is equal to 10 μmol MA/g fish flesh and 10 mmol/kg fish flesh.

Sensory analyses

Six experienced panellists performed sensory analyses. Six trout fillets were reheated in a conventional oven at 75 °C for 3 min before presenting them to the panellists. Sensory evaluation was carried out according to Paulus *et al.* (1979). The panellists evaluated the overall acceptability using a 5-point scale (1, very bad to 5, very good).

Statistical analyses

One-way ANOVA was performed to analyse the chemical parameters and significant differences were determined through the Tukey test. The Kruskal–Wallis test and nonparametric multiple comparisons were performed to determine significant effects of storage period on sensory results. The effects of antioxidant addition on sensory quality were tested applying Mann–Whitney U test. The analyses were performed by SPSS 20 and MS Excell programs.

RESULTS

GC-MS analysis resulted in identification of 34 components for tarragon and 32 components for summer savory EO. Main components of tarragon EO were estragole (81.89%), beta-cis-ocimene (4.62%), beta-trans-ocimene (3.44%), l-limonene (1.67%) and eugenol methyl ether (1.49%); and of summer savory EO were thymol (29.1%), carvacrol (26.6%), gamma-terpinene (24.72%), para-cymene (7.55%) and alpha-terpinene (3.96%).

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Average fat content of the fillets was 2.3 g fat in 100 g fillet. FFA contents of antioxidant treated fillets during the 4-month frozen storage period are shown in Fig. 1. As a result of the cooking procedures, the FFA formation was quite similar in fresh-raw (0.38% oleic acid) and

fried trout fillets ($P>0.05$), but significantly increased in oven baked and steamed fillets ($P<0.05$).

In the present study, the peroxide value (PV, meq/kg) was measured to determine the formation of primary oxidation products. Changes in the PV values

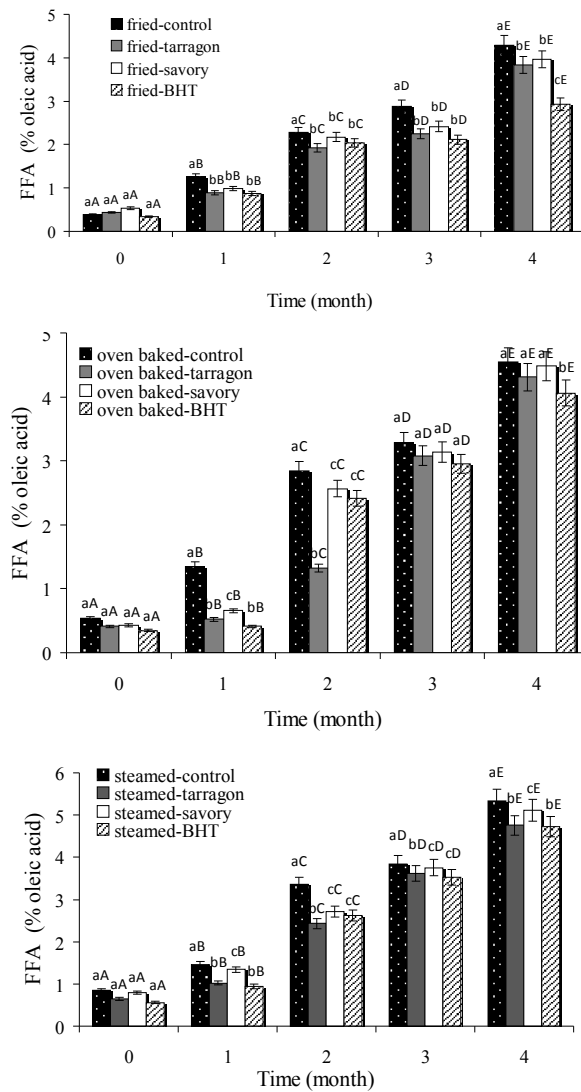


Fig. 1. FFA values (% oleic acid) of precooked trout fillets treated with tarragon and summer savory essential oils during frozen storage. Values (mean \pm SD) with the same superscript (a) within a group and (A) among the groups are not significantly different.

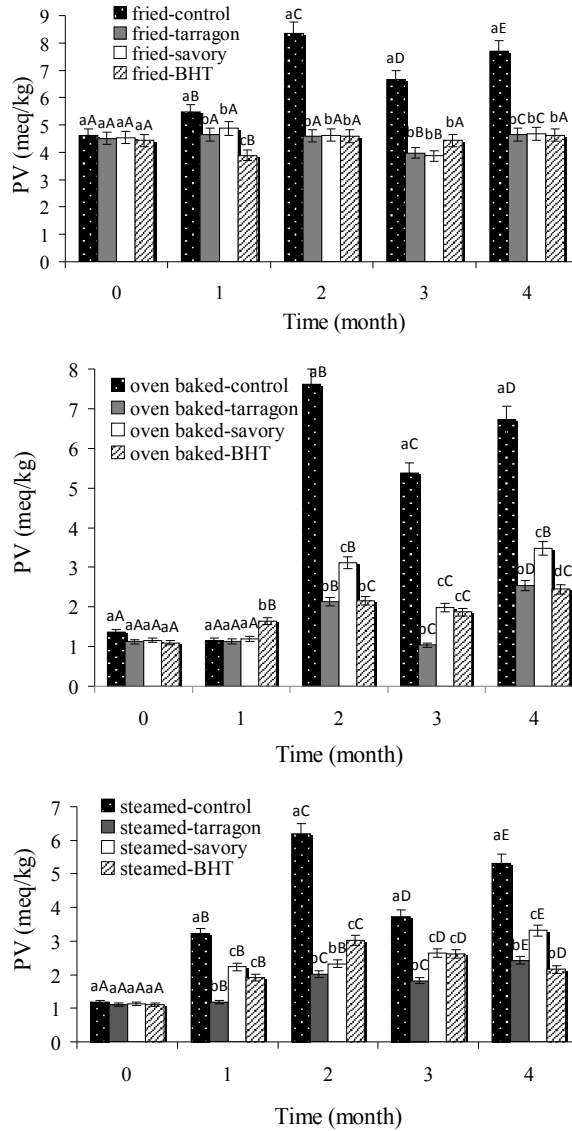


Fig. 2. Peroxide value (PV, meq/kg) of precooked trout fillets treated with tarragon and summer savory essential oils during frozen storage. Values (mean ± SD) with the same superscript (a) within a group and (A) among the groups are not significantly different.

of cooked fillets with tarragon and summer savory EOs during frozen storage are shown in Fig. 2. Initial PV value was 0.093 meq/kg in fresh-raw fillets. The PV values showed an increase in all groups

and the PV of steamed tarragon treated fillets were lower than other samples ($P < 0.05$).

The secondary lipid oxidation products were measured through TBARS

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value, expressed as mg malonaldehyde per kilogram of fish fillets. Changes in the TBARS values of antioxidant treated and cooked trout fillets during frozen storage are shown in Fig. 3. TBARS values in the treated groups (fried, oven baked and steamed, all treated with tarragon and

summer savory EOs) were significantly lower than those of control fillets ($P < 0.05$). Among the treated samples, the highest and the lowest TBARS values were observed in steamed summer savory and oven baked tarragon treated fillets, respectively.

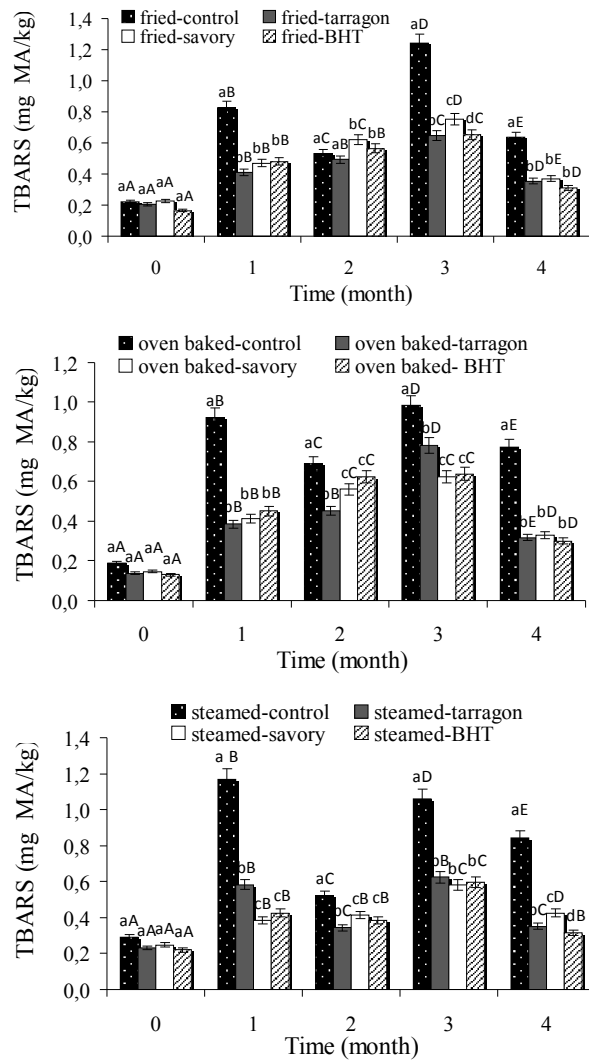


Fig. 3. Thiobarbituric acid reactive substances (TBARS, mg MA/kg) of precooked trout fillets treated with tarragon and summer savory essential oils during frozen storage. Values (mean \pm SD) with the same superscript (a) within a group and (A) among the groups are not significantly different.

Table 1. Overall acceptability scores (median; minimum-maximum) of precooked trout fillets treated with tarragon and summer savory essential oils during frozen storage. All values are mean arithmetic of six observations.

Treatment	Time (month)				
	0	1	2	3	4
Fried – control	4.8 (4.2-5) ^{aA}	4.7 (4.1-4.9) ^{aA}	4.2 (3.8-4.5) ^{bA}	3.6 (3.1-3.9) ^{cA}	2.6 (2.2-3.1) ^{dA}
Fried – tarragon	5 (5) ^{aB}	4.8 (4.3-5) ^{bA}	4.7 (4.4-4.9) ^{bB}	4.6 (4-4.9) ^{cB}	4.5 (3.7-4.8) ^{dB}
Fried – savory	5 (5) ^{aB}	4.7 (4.2-5) ^{bA}	4.6 (4.3-4.8) ^{bC}	4.5 (4.1-4.9) ^{cB}	4.3 (3.8-4.6) ^{dC}
Fried – BHT	4.8 (4.3-5) ^{aA}	4.7 (4.2-5) ^{aA}	4.5 (4.2-4.8) ^{bC}	4 (3.5-4.4) ^{cC}	3.5 (3-4) ^{dD}
Oven baked – control	4.7 (4.3-4.9) ^{aA}	4.6 (4.2-4.8) ^{aB}	3.8 (3-4.4) ^{bD}	3.3 (2.9-3.8) ^{cD}	2.6 (2.2-3) ^{dA}
Oven baked – tarragon	5 (5) ^{aB}	4.8 (4.5-5) ^{bA}	4.6 (4.4-4.8) ^{cC}	4.5 (4.1-4.9) ^{cB}	4.3 (4-4.8) ^{dC}
Oven baked – savory	4.8 (4.3-5) ^{aA}	4.7 (4.5-4.9) ^{aA}	4.6 (4.4-4.7) ^{bC}	4.2 (3.7-4.5) ^{cE}	4.1 (3.6-4.7) ^{cE}
Oven baked – BHT	4.7 (4.5-5) ^{aA}	4.6 (4.3-4.8) ^{aB}	4.2 (3.8-4.5) ^{bA}	3.7 (3.2-4.4) ^{cF}	3.5 (3-3.9) ^{dD}
Steamed – control	4.7 (4.4-4.8) ^{aA}	4.3 (4-4.8) ^{bC}	3.5 (3-4) ^{cE}	3.1 (2.7-4.4) ^{dG}	2.2 (2-2.5) ^{eF}
Steamed – tarragon	4.8 (4.5-5) ^{aA}	4.6 (4.3-4.9) ^{bB}	4.5 (4.1-4.9) ^{bC}	4.3 (4-4.8) ^{cH}	4.2 (3.9-4.6) ^{dC}
Steamed – savory	4.8 (4.5-5) ^{aA}	4.7 (4.5-4.9) ^{aA}	4.3 (4-4.8) ^{bF}	4.2 (3.8-4.5) ^{bE}	4 (3.5-4.5) ^{cE}
Steamed – BHT	4.6 (4.5-4.9) ^{aC}	4.3 (4-4.7) ^{bC}	4.1 (3.6-4.5) ^{cA}	3.8 (3.2-4.1) ^{dF}	3.3 (3-3.8) ^{eG}

^{a/A} Values with the same superscript within the same row (a) or column (A) are not significantly different.

Table 1 shows the changes in the overall acceptability values of cooked trout fillets with and without antioxidants. At the end of the 4-month storage period, a decrease in sensory scores of the all cooked groups was recorded (P<0.05). According to the sensory assessment, generally, all groups were observed to display a decrease from the initial high organoleptic quality (4.5-5) to low quality (<3.5) values at the 4th month of storage. The panelists performing the sensory evalua-

tion determined that the lowest overall sensory acceptability was that of steamed fillets and controls.

DISCUSSION

The main components of the EOs in this study are terpenes, terpenoids and other aromatic and aliphatic constituents. Our results confirm the earlier report of Kordali *et al.* (2005) that major volatile con-

stituents obtained from the aerial parts of *Artemisia dracunculus* L. were anethole (81.0%), beta-ocimene (9.6%), limonene (3.1%) and methyl eugenol (1.8%). Also, Ayoughi *et al.* (2011) reported that the main components of *Artemisia dracunculus* L. were anethole (51.72%), beta-ocimene (8.32%), methyl eugenol (8.06%), limonene (4.94%) and linalool (4.90%). In the present study, the main component of tarragon was estragole (81.89%), an isomer of anethole. The total amount of isomers of beta-ocimene in this study was roughly similar to the results of the research above.

In this work, the main components of summer savory EO were thymol, carvacrol, gamma-terpinene, para-cymene and alpha-terpinene whose high capacity of hydrogen donation, free radicals scavenging and antioxidant activity is shown in several studies (Fathi *et al.*, 2013; Moghadam, 2015). Our results of chemical analysis of summer savory EO were similar to the results of Gulluce *et al.* (2003). In their study, the main components of summer savory EO were reported as thymol (28.9%), carvacrol (26.1%), gamma-terpinene (21.5%), para-cymene (10%), alpha-pinene (3%) and alpha-terpinene (2.7%). The results of Adiguzel *et al.* (2007) showed the major constituents of summer savory EO as thymol (40.54%), gamma-terpinene (18.56%), carvacrol (13.98%) and para-cymene (8.98%). It is suggested that significant antioxidant activity of essential oils in this study is due to the high levels of estragole, thymol and carvacrol as oxygenated monoterpenes and other phenolic compounds that possess high capacity of hydrogen donation.

In the present study, FFA contents increased, especially in the oven baked and steamed samples. In a study conducted by Bakar *et al.* (2008) lipid quality in

cooked, chill-reheated fillets of king mackerel has been investigated. The researchers reported that steaming, grilling, frying and microwave cooking increased the FFA content of mackerel. As the lipolytic enzymes in fish tissue are active even at $-20\text{ }^{\circ}\text{C}$, an increase in the level of hydrolysis of triglycerides and free fatty acid formation can be expected during frozen storage (Hui, 2007).

During heat treatment of food materials lipid oxidation occurs and as a result oxidation products are formed. In this study, the same effect has been observed by all three methods of cooking (frying, oven baking and steaming). Similarly, Tokur *et al.* (2007) reported that peroxide values for rainbow trout (*O. mykiss*) cooked by frying, oven baking and grilling increased. Also, according to the results of Bakar *et al.* (2008) the initial peroxide value (1.78 meq/kg) rose to 2.65, 2.80 and 3.12 meq/kg in fried, grilled and steamed mackerel, respectively. In the present work, a considerable increase in the hydroperoxide formation was observed in all samples during the 4 month frozen storage ($-18\text{ }^{\circ}\text{C}$) period. However, the samples treated with EOs showed lower PV in comparison to the untreated samples. Hydroperoxide formation in sardine (*Sardina pilchardus*) chops treated with rosemary extract during frozen storage was evaluated by Serdaroglu & Felekoglu (2005). The results showed that PV of samples with rosemary extract was significantly lower compared to the control group during storage at $-20\text{ }^{\circ}\text{C}$. It can be concluded that treatments with tarragon and summer savory EOs decreased hydroperoxide formation rate in cooked trout fillets during the frozen storage which is in accordance with the studies above.

As lipid oxidation progresses, the hydroxyperoxides will be degraded into secondary oxidation products with unpleasant sensory effects which have also harmful effects on health due to their neurotoxic, mutagenic and cytotoxic action (Long & Picklo, 2010). Formation of secondary oxidation products (mg MA/kg) from the degradation of hydroxyperoxides in sardine was investigated by Serdaroglu & Felekoglu (2005). They reported that control samples were more rancid than those treated with rosemary extract during the storage time at -20°C . In a study by Tokur *et al.* (2007), it was found that initial TBARS value (0.22 MA/kg) of rainbow trout (*O. mykiss*) muscle cooked by oven baking increased to 5.78 mg MA/kg and that heating such as smoking, grilling and frying led to an increase of TBARS. Also, Bakar *et al.* (2008) reported a raise in the initial TBARS value (0.54 mg MA/kg) of raw muscles to 2.98, 2.80, 3.13 and 2.65 mg MA/kg after applying several cooking methods, frying, grilling, steaming and microwave heating, respectively. The results of the above studies are in accordance with our work; however, in the present research TBARS in steamed samples (with and without antioxidant) were higher than the samples cooked by other methods ($P < 0.05$).

TBARS values in all groups in this study showed fluctuation, as seen in Fig. 3. The decreased TBARS content could be a result from the formation of lipid oxidation products, and the interaction of the present malonaldehyde with the other compounds, myofibrillar protein in particular (Melton, 1983). In general, it could be concluded that treatment of trout fillets with tarragon and summer savory EOs exerted considerable effects in preventing malonaldehyde formation in comparison to control group.

From the results of the sensory analysis, fried fillets were the most acceptable, scoring highest in overall acceptability. Steamed fillets were the least acceptable as they had the lowest scores for overall acceptability. In general the overall range in mean sensory assessment scores was extensive. The maximum range was 5 on a five-point scale for fried fillets without antioxidant. Dreeling *et al.* (2000) studied the effect of cooking methods, such as grilling, frying, griddling and roasting, on the quality of low-fat beef burgers. They found that griddled burgers had the highest scores for overall acceptability. In contrast to our results, these researchers observed a low range in sensory scores of the beef burgers. Similarly, Tokur *et al.* (2007) found that sensory scores of cooked carp finger (180°C for 30 s) decreased during the 5-month frozen storage period but they were all still within acceptable range. There was no considerable difference between the sensory scores of tarragon treated and summer savory treated fillets at the beginning of the study (just after cooking) and during the frozen storage period. However, overall acceptability score of antioxidant treated samples was higher than control. The acceptability of fishery products during storage in frozen condition depends on the changes of their sensory characteristics. The data obtained from this study also showed that fried and oven baked fillets of trout could be stored at -18°C for 4 months while maintaining their acceptable quality in terms of sensory analysis. The results obtained from chemical quality analyses confirms these conclusions.

The results of the present study show that treating fish fillets with tarragon and summer savory EOs before cooking reduces the rate of lipid oxidation (tarragon EO showing higher antioxidant activity

than summer savory EO). It also can be concluded that these EOs retarded lipid oxidation of cooked trout fillets during the frozen storage. The acceptability scores of all cooked groups decreased during the 4 months of frozen storage, however, EO-treated fried and oven baked trout fillets could be stored at -18°C for 4 months, maintaining acceptable sensory quality.

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