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ORIGINAL PAPER

# Effect of the Fumigating with Essential Oils on the Microbiological Characteristics and Quality Changes of Refrigerated Turbot (*Scophthalmus maximus*) Fillets

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**Abstract** Antimicrobial activity of essential oils is widely known, but their application to fish preservation is already limited. The objective was to evaluate the microbiological characteristics and quality changes in turbot (*Scophthalmus maximus*) fillets with essential oil fumigation treatments over 20 days stored at 2±1 °C. The turbot fillets were fumigated with a series of concentrations (1, 4, and 8 μL/L) of essential oils of clove, cumin, and spearmint. Changes in the color, texture profile analysis (TPA), peroxide value (PV), 2-thiobarbituric acid (TBA), total volatile basic nitrogen (TVB-N), trimethylamine nitrogen (TMA-N), microbial characteristics (total viable count, psychrotrophic count, pseudomonads, *Shewanella putrefaciens*, Enterobacteriaceae, and lactic acid bacteria count) were measured. The results showed the turbot fillets from the control group were the first (day 10) to indicate signs of degradation reaching rejection threshold values for all evaluated parameters. All essential oils could inhibit the oxidation of turbot fillets, and the most effective essential oil was spearmint oil. Fumigated with 4 μL/L spearmint oil maintained the color and texture, retarded the lipid and protein oxidation, and reduced the microorganism counts. Therefore, post-mortem essential oil fumigation treatment has positive effects on improving the quality of refrigerated turbot fillets.

**Keyword** Turbot · Essential oil · Microbiological control · Quality change · Fumigation

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## Introduction

The flatfish turbot (*Scophthalmus maximus*), as a commercially important marine fish species, is native to Europe and introduced to China in 1990s. Nowadays, China becomes the largest producer of turbot in the world and the yield of turbot reached 113,551 t in 2013, largely due to the genetic breeding program in turbot: increasing growth rate, controlling sex ratio, and improving disease resistance (Imsland and Jonassen 2001). The turbot is highly appreciated for its nutritional properties and is highly popular with consumers. The turbot is traditionally sold as a whole and stored in ice, but is hard to handle or to prepare due to the length and weight. For much more convenient sales and consumption, the fresh turbot fillets have already become a popular product in the supermarket. However, the commercial value of turbot fillets was lost within 10 days in air packaging at 2±1 °C due to the endogenous enzyme and microbial attack (Santos et al. 2013). The short shelf life of the turbot fillets is an impediment to the distribution and marketing of the fresh sea product. Thus, prolonging the shelf life of turbot fillets, while preserving their quality, would benefit the turbot industry as well as consumers.

Essential oils are aromatic oily extracts that can be obtained by the method of steam distillation from plant materials, such as leaves, seeds, flowers, buds, roots, and other plant parts (Burt 2004). As an alternative to chemical and synthetic preservatives, essential oils can be used in any food, meet

the demands of consumers for natural products and are generally recognized as safe (USFDA 2006). Several studies have shown that essential oils have marked antimicrobial activities in fish (Harpaz et al. 2003; Mahmoud et al. 2004; Coban 2013; Daniel et al. 2014). The addition of laurel, cumin, or oregano essential oil in the surface of sea bream fillets induced a decrease in lipid oxidation (Golas and Kontominas 2007; Attouchi and Sadok 2012). The edible films incorporated with thyme or oregano essential oil extended the shelf life of refrigerated rainbow trout fillets (Gomez-Estaca et al. 2010; Jouki et al. 2014). Carvacrol essential oil released from the active package could protect the salmon cubes from spoilage by food-borne microorganisms (Cerisuelo et al. 2013). Additionally, Gao et al. (2014) reported that microorganism counts in button mushrooms during refrigerated storage were reduced by essential oils fumigation treatment from clove, cinnamon, and thyme. However, to our knowledge, little information is available on the response of turbot fillets to essential oil fumigation treatment.

Thus, this study was performed to investigate the effect of essential oils (clove, cumin, and spearmint) fumigation treatment on turbot fillets quality changes over 20 days chilled storage. Parameters related to physicochemical responses and microbiological characteristics, such as color, texture profile analysis (TPA), peroxide value (PV), 2-thiobarbituric acid (TBA), total volatile basic nitrogen (TVB-N), and trimethylamine nitrogen (TMA-N), total viable count (TVC), psychrotrophic count (PTC), pseudomonads, and *Shewanella putrefaciens*, Enterobacteriaceae, lactic acid bacteria (LAB) were evaluated at pre-storing (0), 5, 10, 15, and 20 days of storage, respectively.

## Materials and Methods

### Preparation of Sample and Treatment

Fresh turbot (*S. maximus*) with a mean weight and length of 0.74±0.08 kg and 31.72±0.24 cm were obtained from a local aquaculture farm in Jinzhou, China. Fish were directly transported to the Aquatic Products Processing Laboratory of Bohai University within 2 h. The turbot were decapitated and filleted by hand, and the fillets were immediately treated for essential oil fumigation. In a preliminary experiment, we measured a series of concentrations of each essential oil, including clove, cumin, and spearmint, that is, 1, 4, and 8 μL/L. All essential oils at the concentration of 1 or 4 μL/L significantly inhibited fillets spoilage, and 4 μL/L had the better effect. However, 8 μL/L of essential oil fumigation treatment caused some sensory damages, including off-flavor or discoloration in the turbot fillets. Therefore, a concentration of 4 μL/L was chosen to use in this experiment. For each treatment, 20 fillets with an average weight of 100 g were used. Four fillets each were placed on the shelf in a 10-L sealed polypropylene container, with a filter paper inside the cover. Forty microlitres of each essential oil, including clove, cumin, and spearmint, was spotted on to the filter paper. Then these containers were placed at 10 °C, and the essential oils were allowed to vaporize within the containers for 2 h. After that, the fillets were packed in polyethylene bags and stored in refrigerated chambers at 2±1 °C during 20 days for quality analysis, and subsequently every 5 days, three replicates from each group were analyzed.

### Color Measurement

The surface color of turbot fillets was measured with a WSC-S colorimeter (Shanghai Precision Instrument Co. Ltd., Shanghai, China). To analyze the $L^*$ (black/white), $a^*$ (red/green), and $b^*$ (yellow/blue) values, three measurements were taken for each fillet. The color intensity is expressed by a chroma value ($C^*ab$), while hue ($H^0ab$) represents the purity of color, were calculated according to the equation (Palou et al. 1999): $C^*ab=(a^{*2}+b^{*2})^{1/2}$ and $H^0ab=\arctan(a^*/b^*)$, respectively.

### Texture Profile Analysis (TPA)

The texture properties of fish fillets were evaluated at room temperature using a TA-XT plus texture analyzer (Stable Micro Systems Ltd., Godalming, UK) equipped with a 5-mm diameter cylindrical probe (P/5). Texture profile analysis (TPA) was performed using the dorsal muscle of fillet (1.5×1.5×1.0 cm) which was compressed twice to 75 % of its original height. The speed of probe was 2 mm $s^{-1}$ during penetration. The parameters (hardness, cohesiveness, adhesiveness, springiness, chewiness, gumminess, and resilience) were calculated from published definitions (Bourne 2002). Three replicates were made for each test sample.

### Peroxide Value (PV) and Thiobarbituric Acid (TBA) Value

The peroxide value was expressed as meq/kg fat, was determined by iodometric titration after an addition of acetic acid (AOAC 2005). Homogenized samples (2 g) were dissolved into a 250-mL stoppered iodine flask containing 30 mL of mixture of chloroform and acetic acid (2:3, *v*/*v*). Then 1 mL of potassium iodide saturated solution was added, and the mixture was shaken for 0.5 min. The flask was kept for 3 min in the dark before 100-mL distilled water was added. Potassium iodate was assayed by gravimetric titration with the sodium thiosulfate solution. Analyses were conducted in triplicate and all reagents were of analytical grade.

In this study, the TBA value of fish samples was evaluated by measuring the concentration of malonaldehyde (Botsoglou et al. 1994) with some modification. Samples (200 mg) were homogenized with 4.8 mL of a 5 % solution of potassium

chloride. To 0.5 mL of homogenate, 3 mL of 1 % phosphoric acid and 1 mL of 0.6 % TBA aqueous solution were added. The mixture was incubated in boiling water for 90 min followed by an ice bath for 10 min. Then 4 mL of 1-butanol was added. The tubes were shaken and the supernatant was removed after centrifugation. The absorbance (As) of the resulting pigment was recorded at 532 nm using a UV-vis spectrophotometer (UV-2550, Shimadzu). A reagent blank was run and the absorbance (Ab) recorded. Three replicates were made for each test sample and the absorbance values were converted to the TBA value (mg of malonaldehyde equivalents/kg of tissue) using Eq. (1):

$$\mathrm{TBA} = 50 \times (\mathrm{As}-\mathrm{Ab})/200 \quad (1)$$

### Total Volatile Basic Nitrogen (TVB-N) and Trimethylamine Nitrogen (TMA-N)

TVB-N values were determined as described by Ozogul and Balikci (2013) with a Kjeltec 8400 (Foss, Sweden) using steam distillation for extraction of volatile bases from fish samples. Briefly, 10 g of fish flesh from a mixture of both fillets was homogenized with 50 mL of distilled water on a Kjeldahl distillation tube. After homogenization, 3 mL of silicone antifoaming agent and 1 g of MgO were added. The distillate was collected into 10 mL of 0.1 M hydrochloric acid solution with an indicator solution (methyl red). Steam distillation process was ended after check with a pH strip for the complete absence of alkalinity on the distillate. The distillate was titrated with 0.0167 M sodium hydroxide solution, and the results were expressed in milligram nitrogen per 100-g sample. Analyses were conducted in triplicate and all reagents were of analytical grade.

TMA-N was determined according to the official procedure of the AOAC (2005). Homogenized samples (10 g) were weighed and blended with 90 mL of 7.5 % trichloroacetic acid solution and then filtered. Four milliliters of extract was transferred into test tubes, and then 1 mL of 20 % formaldehyde, 10 mL of anhydrous toluene, and 3 mL of $K_2CO_3$ saturated solution were added. The tubes were shaken, and then the 5-mL toluene layer was pipetted. Five milliliters of 0.02 % picric acid solution was added. The absorbance was recorded at 410 nm against a blank control, and the TMA-N value was expressed as milligrams per 100 g of muscle. Three replicates were made for each test sample.

### Microbiological Characteristics

The fillet samples (25 g) were obtained aseptically and transferred to 225 mL of sterile 0.1 % peptone water solution. The mixture was homogenized for 60 s using a BagMixer (Model 400; Interscience, France). For microbial count, 0.1 mL samples of serial dilutions (1:10) of flesh homogenates were spread on the plates of various agar materials. Six serial decimal dilutions were applied for microbiological evaluation of fillet samples. TVC was determined on plate count agar (PCA; Aoboxing Bio-Tech, Beijing, China) by counting the number of colony-forming units after incubation at 35 °C for 48 h. PTC was performed on PCA after incubation at 7 °C for 10 days. Pseudomonad growth was determined on cephaloridin fucidin cetrimide agar (Aoboxing Bio-Tech, Beijing, China) and incubated at 30 °C for 48 h. *S. putrefaciens* were counted from the black colonies grown on iron agar (Aoboxing Bio-Tech, Beijing, China) at 20 °C for 72 h, and a representative number of colonies were confirmed by using API 20NE (Biomerieux, France). Enterobacteriaceae were enumerated in violet red bile glucose agar (Aoboxing Bio-Tech, Beijing, China) with a double layer at 30 °C for 24 h. LAB were enumerated on de Man Rogosa Sharpe agar (Aoboxing Bio-Tech, Beijing, China) incubated at 25 °C for 5 days under anaerobic conditions. Three replicates were made for each sample and four appropriate dilutions were used for each replicate. Microbiological data were transformed into logarithms of the number of colony forming units (CFU/g).

### Statistical Analysis

The experiment followed a completely randomized design ($n$=3). Data were subjected to two-way analysis of variance (ANOVA). Mean separations were assessed by Duncan's multiple range test (SAS version 8.1). Differences at $p<0.05$ were considered significant.

## Results and Discussion

### Effect of Essential Oils Fumigation on Color

The appearance of seafood is of major importance to consumers, both from the point of view of acceptability and preference. Thus, color plays a decisive role when evaluating the quality of the product at the point of sale. The color is influenced by both muscle structure characteristics and pigment concentrations (Gines et al. 2004). Changes in the external color were monitored by determining $L^*$, $a^*$, $b^*$, $C^*ab$, and $H^0ab$ values. Table 1 shows the different values obtained after application of essential oils fumigation. From this table, the $L^*$ value of the control sample decreased sharply, higher $L^*$ values ($p<0.05$) were observed in essential oils fumigated samples compared to control after 10 days of storage. At the end of the storage, the treatment with spearmint did not cause the same effect as the treatment with clove, but cumin and

**Table 1** Effect of essential oil fumigation treatment on color parameters evolution ($L^*$, $a^*$, $b^*$, $C^*ab$, and $H^0ab$) of turbot fillet stored at 2 °C for 20 days

| Days at 4 °C | 0 | 5 | 10 | 15 | 20 |
|---|---|---|---|---|---|
| $L^*$ | | | | | |
| Control | 63.62±0.83 aA | 61.78±1.31 aA | 57.01±0.76 bB | 54.81±0.88 cC | 52.80±1.43 cD |
| Clove | 63.38±0.42 aA | 61.74±0.66 aB | 58.60±0.64 abC | 56.82±1.38 bD | 55.60±0.19 bD |
| Cumin | 63.61±0.66 aA | 62.10±1.11 aA | 58.95±1.16 aB | 57.71±0.48 abBC | 56.52±0.78 abC |
| Spearmint | 63.52±1.32 aA | 62.66±0.67 aA | 60.27±1.06 aB | 59.31±0.91 aBC | 58.14±1.37 aC |
| $a^*$ | | | | | |
| Control | 1.53±0.15 aA | 1.44±0.08 aAB | 1.32±0.06 aBC | 1.20±0.06 aC | 1.32±0.09 aBC |
| Clove | 1.58±0.16 aA | 1.34±0.23 aAB | 1.36±0.21 aAB | 1.25±0.11 aAB | 1.17±0.12 aB |
| Cumin | 1.56±0.11 aA | 1.35±0.10 aBC | 1.45±0.09 aAB | 1.30±0.04 aBC | 1.23±0.05 aC |
| Spearmint | 1.56±0.08 aA | 1.41±0.11 aAB | 1.32±0.10 aAB | 1.34±0.23 aAB | 1.27±0.03 aB |
| $b^*$ | | | | | |
| Control | −6.64±0.27 aE | −5.07±0.30 aD | −4.19±0.55 aC | −2.17±0.56 aB | −0.80±0.34 aA |
| Clove | −6.76±0.16 aE | −5.75±0.46 abD | −4.95±0.36 bC | −3.46±0.23 bB | −1.56±0.26 bA |
| Cumin | −6.89±0.16 aE | −6.04±0.28 bD | −5.38±0.29 bC | −3.49±0.66 bB | −2.09±0.14 cA |
| Spearmint | −6.86±0.07 aD | −5.83±0.41 bC | −5.63±0.23 bC | −3.88±0.33 bB | −2.62±0.15 dA |
| $C^*ab$ | | | | | |
| Control | 6.81±0.27 aA | 5.27±0.29 bB | 4.40±0.53 bC | 2.49±0.48 bD | 1.57±0.15 dE |
| Clove | 6.94±0.13 aA | 5.91±0.43 abB | 5.14±0.40 aC | 3.68±0.19 aD | 1.96±0.16 cE |
| Cumin | 7.06±0.18 aA | 6.19±0.28 aB | 5.57±0.29 aB | 3.73±0.61 aC | 2.43±0.14 bD |
| Spearmint | 7.03±0.06 aA | 6.00±0.39 aB | 5.79±0.25 aB | 4.11±0.32 aC | 2.91±0.14 aD |
| $H^0ab$ | | | | | |
| Control | −0.23±0.02 aA | −0.28±0.02 aA | −0.31±0.03 bA | −0.52±0.12 bB | −1.04±0.21 bC |
| Clove | −0.23±0.03 aA | −0.23±0.05 aA | −0.27±0.03 aAB | −0.35±0.05 aB | −0.65±0.12 aC |
| Cumin | −0.22±0.01 aA | −0.22±0.01 aA | −0.26±0.01 aA | −0.36±0.07 aB | −0.53±0.01 aC |
| Spearmint | −0.22±0.01 aA | −0.24±0.03 aA | −0.23±0.01 aA | −0.33±0.06 aB | −0.45±0.02 aC |

All values were means±standard deviation of three values

Different small letters in the same column indicate significant differences between means ($p<0.05$)

Different capital letters in the same row indicate significant differences between means ($p<0.05$)

spearmint treatments showed similar $L^*$ values during storage. Compared with control, essential oils fumigation treatments can inhibit the darkening of the turbot flesh. There is no evidence of the role of these natural compounds on this issue, but the well known antioxidant activity reported for these essential oils may retard the oxidation of lipids and proteins (Sowbhagya 2013; Bensid et al. 2014; Kedia et al. 2014) and thereby maintain the appearance of fish samples. The parameters of $a^*$ and $b^*$ are more related to the color perceived by the human eye. Value of $b^*$ showed a similar trend with lipid oxidation such as MDA or PV, and evolved to a more yellowish color during storage. Similar result was obtained in another research, in which the $b^*$ value increased under the gelatin films with laurel leaf essential oil treatment in rainbow trout stored at 4 °C for 26 days (Alparslan et al. 2014). Regarding coordinates $a^*$, no significant differences ($p>0.05$) were observed over the period of storage in the present study and no significant differences in $a^*$ values were observed in samples treated with essential oils compared to control sample. There were no significant differences for $C^*ab$ and $H^0ab$ values of samples between different essential oil treatments during the first 15 days of storage. However, the samples exposed to essential oil fumigation with spearmint had higher $C^*ab$ value ($p<0.05$) at the end of storage.

## Effect of Essential Oils Fumigation on Texture Profiles

Texture profile analysis (TPA) was performed to determine the effect of essential oils fumigation on the texture of turbot fillets and reflected freshness changes and changes in water content (Cai et al. 2014). The physical properties (hardness, springiness, gumminess, chewiness, cohesiveness, adhesiveness, and resilience) for textural evaluation are shown in Table 2. The initial hardness values of treated and control samples were similar, but clearly, the control sample had the fastest softening rate, losing about 49.18 % of its hardness over 20 days of storage. The hardness of samples treated with the clove, cumin, and spearmint fumigation also decreased but

**Table 2** Effect of essential oil fumigation treatment on texture profiles (hardness, springiness, chewiness, gumminess, cohesiveness, adhesiveness, and resilience) of turbot fillet stored at 2 °C for 20 days

| Days at 2 °C | 0 | 5 | 10 | 15 | 20 |
|---|---|---|---|---|---|
| Hardness (N) | | | | | |
| Control | 170.68±8.58 aA | 143.40±4.25 bB | 128.51±5.28 bC | 114.42±7.57 cD | 86.74±6.65 cE |
| Clove | 169.82±9.04 aA | 158.49±4.86 aA | 144.06±9.39 aB | 129.90±2.68 bC | 112.24±4.39 bD |
| Cumin | 169.88±4.57 aA | 155.78±8.74 aB | 148.15±5.14 aB | 132.45±6.54 abC | 119.74±2.48 bD |
| Spearmint | 170.81±3.49 aA | 162.27±3.42 aAB | 153.55±6.01 aB | 142.68±4.58 aC | 132.46±5.82 aD |
| Springiness (mm) | | | | | |
| Control | 0.95±0.02 aA | 0.89±0.02 aB | 0.83±0.02 aC | 0.71±0.03 cD | 0.57±0.03 bE |
| Clove | 0.94±0.01 aA | 0.91±0.02 aA | 0.84±0.03 aB | 0.74±0.01 bcC | 0.69±0.05 aD |
| Cumin | 0.94±0.03 aA | 0.91±0.03 aA | 0.86±0.02 aB | 0.78±0.03 abC | 0.68±0.03 aD |
| Spearmint | 0.95±0.01 aA | 0.93±0.02 aA | 0.87±0.02 aB | 0.81±0.02 aC | 0.73±0.02 aD |
| Chewiness (N mm) | | | | | |
| Control | 107.19±9.22 aA | 90.32±4.38 bB | 74.95±2.08 bC | 61.58±2.03 cD | 43.95±11.29 bE |
| Clove | 108.57±6.85 aA | 97.23±6.15 abB | 82.92±1.48 aC | 69.98±3.05 abD | 58.71±3.22 aE |
| Cumin | 107.98±8.06 aA | 95.97±3.84 abB | 84.28±2.95 aC | 65.31±5.25 bcD | 57.94±3.20 aD |
| Spearmint | 108.13±6.98 aA | 100.42±3.82 aA | 87.83±5.11 aB | 74.41±0.81 aC | 60.04±2.57 aD |
| Gumminess (N) | | | | | |
| Control | 124.11±10.58 aA | 95.50±2.54 abB | 71.57±3.57 bC | 59.86±6.54 bD | 46.01±3.19 cE |
| Clove | 124.14±5.82 aA | 96.68±2.92 abB | 83.05±7.20 aC | 74.99±2.23 aC | 56.10±2.91 bD |
| Cumin | 125.07±8.69 aA | 90.71±4.13 bB | 82.38±7.71 aBC | 77.31±2.19 aC | 60.95±3.54 bD |
| Spearmint | 125.98±8.54 aA | 99.57±5.86 aB | 85.64±1.68 aC | 80.01±3.34 aC | 68.75±4.60 aD |
| Cohesiveness | | | | | |
| Control | 0.35±0.04 bA | 0.38±0.03 aA | 0.37±0.05 aA | 0.40±0.03 aA | 0.37±0.02 aA |
| Clove | 0.40±0.02 aA | 0.37±0.01 abAB | 0.35±0.02 aB | 0.36±0.03 aB | 0.33±0.02 aB |
| Cumin | 0.36±0.01 abA | 0.35±0.02 bA | 0.39±0.02 aA | 0.38±0.07 aA | 0.34±0.03 aA |
| Spearmint | 0.38±0.01 abAB | 0.36±0.02 abAB | 0.40±0.04 aA | 0.34±0.02 aB | 0.37±0.02 aAB |
| Adhesiveness (Ns) | | | | | |
| Control | −14.27±0.85 bC | −12.33±0.97 aAB | −10.83±1.35 aA | −12.75±0.80 aBC | −13.44±0.81 aBC |
| Clove | −11.76±1.37 aAB | −10.46±1.23 aA | −14.77±3.25 bBC | −13.87±1.79 aABC | −15.69±1.42 bC |
| Cumin | −14.49±1.74 bAB | −16.06±2.29 bB | −13.55±0.84 abAB | −12.13±0.50 aA | −15.19±1.35 abB |
| Spearmint | −13.89±0.66 abAB | −12.93±1.36 aA | −15.12±1.06 bB | −13.90±1.26 aAB | −14.14±0.24 abAB |
| Resilience (mm) | | | | | |
| Control | 0.17±0.02 aA | 0.15±0.04 aA | 0.13±0.02 abA | 0.15±0.02 aA | 0.16±0.02 aA |
| Clove | 0.16±0.02 aA | 0.13±0.01 aAB | 0.11±0.02 bB | 0.14±0.04 aAB | 0.15±0.02 aAB |
| Cumin | 0.18±0.02 aA | 0.13±0.01 aB | 0.14±0.02 abB | 0.13±0.01 aB | 0.13±0.03 aB |
| Spearmint | 0.17±0.03 aA | 0.14±0.01 aAB | 0.16±0.02 aAB | 0.12±0.02 aB | 0.13±0.02 aB |

All values were means±standard deviation of three values

Different small letters in the same column indicate significant differences between means ($p<0.05$)

Different capital letters in the same row indicate significant differences between means ($p<0.05$)

to a lesser extent, 33.91, 29.51, and 22.45 %, respectively, which was lower than that of control. The lowest reduction of fish flesh in hardness was obtained for those treated with spearmint. Similar changes were also observed in springiness, chewiness, and gumminess parameters. Decrease in hardness, springiness, chewiness, and gumminess of turbot flesh during storage primarily due to both the action of endogenous enzymes and microbial activity in fillets, the former consisted of calpains, cathepsins, and collagenases, resulting the degradation of proteins, and the latter thereafter was accelerated along with the breakdown of proteins (Cai et al. 2014). In the present study, essential oil fumigation treatment groups had no significant differences ($p>0.05$) compared to control sample in cohesiveness, adhesiveness, and resilience during storage. Feng et al. (2012) found that texture properties (especially hardness, gumminess, and chewiness) of black sea bream

were closely related with microbial activity, which was in agreement with our results. In the present study, texture properties could be delayed for deterioration by essential oil fumigation treatments during refrigerated storage.

## Effect of Essential Oils Fumigation on PV and TBA Value

Lipid oxidation is one of the primary causes of quality deterioration of fish and fish products. It depends on many elements, such as fish species, lipid content, storage conditions, and so on (Serdaroglu and Felekoglu 2005). Rancidity development in fish and fish products can be followed by determining the increase in hydroperoxides (PV value) and by measuring the formation of malondialdehyde (TBA value). Figure 1a shows the variation in the PV value of turbot fillets stored at 2 °C under the three treatments. The PV value of all samples (clove, cumin, spearmint, and control) gradually increased throughout the whole storage period, reaching 3.32, 3.27, 3.01, and 5.36 meq/kg of lipids, respectively. The results indicated that essential oils fumigation treatments were effective in retarding the production of PV in turbot fillets stored by refrigeration. Coban (2013) also reported that essential oils including sage, thyme, and clove oils, functioned in suppressing lipid oxidation in rainbow trout fillet. There were no significant differences ($p>0.05$) between essential oil fumigation treatments. However, lower PV values in fumigated samples were found compared to control sample, which might be explained by the antioxidant action of essential oils in the lipid oxidation during storage. In the present study, the PV value gave an indication on behave of the first stage of oxidative rancidity. Accordingly, the change trend of PV value did not reflect the expected decomposition of peroxides to secondary products which characterize the later stage of lipid oxidation (Ross and Smith 2006).

Figure 1b shows changes in the TBA value of turbot fillets with different treatments during 20 days of storage. The initial TBA value of the turbot was 0.16 mg MDA/kg muscle. Although the TBA values of both treated and control samples

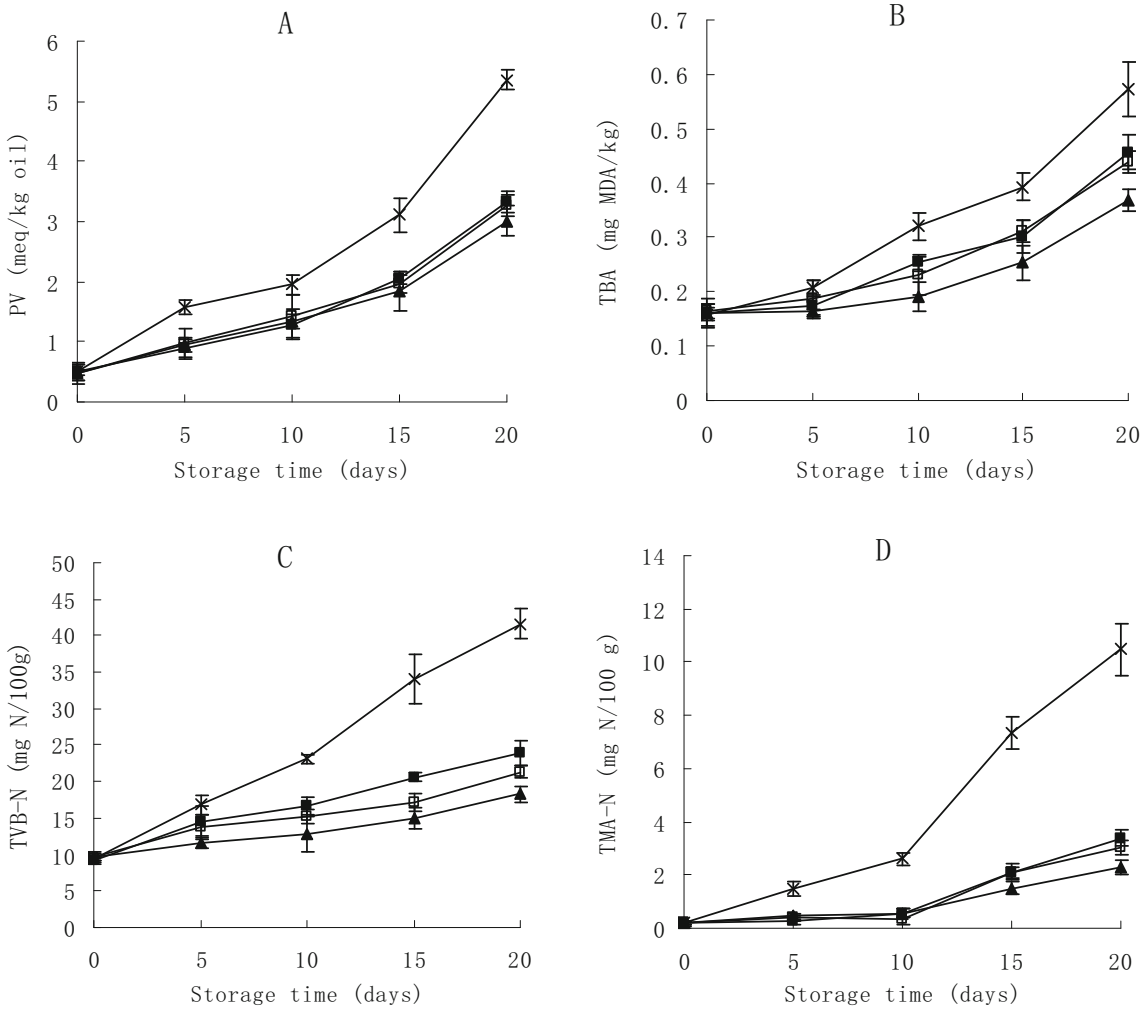


**Fig. 1** Changes in **a** PV, **b** TBA, **c** TVB-N, and **d** TMA-N of turbot fillets treated with control (×), clove (■), cumin (□), and spearmint (▲) stored at 2 °C for 20 days. Each data point is the mean of three replicate samples. *Vertical bars* represent standard deviation of means

increased during storage, the uses of clove and cumin oils fumigation significantly ($p<0.05$) retarded the increase of TBA values in turbot samples, reaching 0.25 and 0.23 mg MDA/kg muscle on day 10, respectively, compared to the control sample (0.32 mg MDA/kg). In contrast, a relatively smaller increase in the TBA value (0.19 mg MDA/kg) of the sample fumigated with spearmint was recorded. TBA values obtained after the fumigation of clove, cumin, and spearmint oils were 56, 44, and 19 % higher than the initial value on day 10, respectively. After 20 days of storage, the TBA values of turbots treated with clove, cumin, and spearmint was 0.46, 0.44, and 0.37 mg MDA/kg, respectively, whereas the control sample attained 0.57 mg MDA/kg. In the present study, both PV and TBA values in turbot fillets decreased by the treatments with different essential oils, indicating the primary and secondary processes were all substantially inhibited. These were consistent with the findings by Attouchi and Sadok (2012) in sea bream, Taheri et al. (2013) in cobia fish fillets, and Alparslan et al. (2014) in rainbow trout fillets.

## Effect of Essential Oils Fumigation on TVB-N and TMA-N

TVB-N as a parameter could quantify the compounds consisted of ammonia and primary, secondary, and tertiary amines, and is usually used as an indicator of deterioration of muscle tissues (Ozogul and Balikci 2013). As shown in Fig. 1c, the TVB-N values increased in all samples during storage. A variety of researchers have reported that the acceptable limit for fresh fish was 30 mg TVB-N of flesh (El-Marrakchi et al. 1990; Harpaz et al. 2003; Bensid et al. 2014). The initial TVB-N values (9.24–9.77 mg N/100 g muscle) indicated that the turbot fillets were of good quality, in agreement with the relatively low initial TVC count (2.76–2.87 log10 CFU/g). The initial TVB-N value in the present study was lower than that reported by Santos et al. (2013) for turbot fillet (>10 mg N/100 g muscle). The TVB-N is a product of bacterial spoilage and endogenous enzymes action in fish and fish products. The strong digestive enzymes may cause rapid post-mortem autolysis during the later phase of storage, followed by a strong off-flavor, which is closely related to the decomposition of protein and the production of volatile nitrogen compositions (Orban et al. 2011). The lower TVB-N values ($p<0.05$) of samples fumigated with essential oils may be attributed to the antibacterial properties of the phenolic constituents in essential oils (Attouchi and Sadok 2012; Bensid et al. 2014). In the current study, the TVB-N value for control turbot fillet stored in refrigerated condition exceeded limit of acceptability of 30 mg/100 g muscle on the 15th day. The TVB-N values for turbot fillets fumigated with clove, cumin, and spearmint were remained below the upper limit of acceptability till the storage ended, values were 24.01, 21.36, and 18.27 mg N/100 g for samples fumigated with clove, cumin, and spearmint oils, respectively. Lower TVB-N value ($p<0.05$) in the spearmint group was found in comparison to turbot fillet stored in the clove or cumin group. The above results indicated that the TVB-N is a suitable index for the spoilage of turbot fillets stored at 2 °C.

The TMA-N changes in turbot fillets during refrigerated storage are presented in Fig. 1d. The TMA-N as a parameter to be monitored in the quality control of seafood products, but no official limit has been established yet. A level of 5 mg TMA-N/100 g of fish flesh has been considered spoiled and unfit for human consumption (Attouchi and Sadok 2012). The TMA-N increased very slowly from day 0 to day 10 in treated and control samples. Although the TMA-N in control sample increased sharply from day 10 to end exceeding the regulation limit, the samples fumigated with essential oils were still below the 5 mg N/100 g when the storage period stopped. The TMA-N contents determined in the present study were remarkably low in essential oil fumigation treatments, suggesting a very limited growth of TMA-producing bacteria in the treated samples, such as *S. putrefaciens*; this result that would be confirmed for turbot fillet by microbiological analysis, as described subsequently. Thus, it can be stated that essential oils inhibit bacterial growth and reduce the accumulation of the TMA, resulting in an extension of the shelf life of fish fillets. From the results obtained, the development of volatile amines, in particular the TMA-N, can be concluded to be an accurate index for the evaluation of quality changes.

## Effect of Essential Oils Fumigation on Microbiological Characteristics

Microbiological characteristics of turbot fillets fumigated with essential oils during storage at 2 °C for 20 days are shown in Fig. 2a–f. The TVC exhibited slower growth rates in samples fumigated with clove oil and cumin oil due to their high antimicrobial activities compared to the control sample. The control sample after 10 days of storage showed a TVC higher than 7 log CFU/g, the recommended acceptable limit for the fish and fish products (ICMSF 1986). As a result of muscle contamination in handling process of fillets, it was expected to have a shorter shelf life than the whole fish stored in ice (Aubourg et al. 2005; Nuin et al. 2008; Cai et al. 2014). The sample fumigated with spearmint oil possessed the lowest TVC in all treated samples when the storage period ends, indicating a better antimicrobial effect of spearmint oil ($p<0.05$) compared with that of clove oil. Psychrotrophic bacteria cause most of changes in odor and flavor as a result of production of different metabolic compounds such as aldehydes, ketones, volatile sulfides, and biogenic amines (Ojagh et al. 2010). The initial PTC of turbot fillet was 2.29 log CFU/g, which rapidly increased to 3.65 and 6.28 log CFU/g for control samples on day 5 and day 15, respectively. The use of essential oils fumigation in turbot fillets also reduced the PTC.

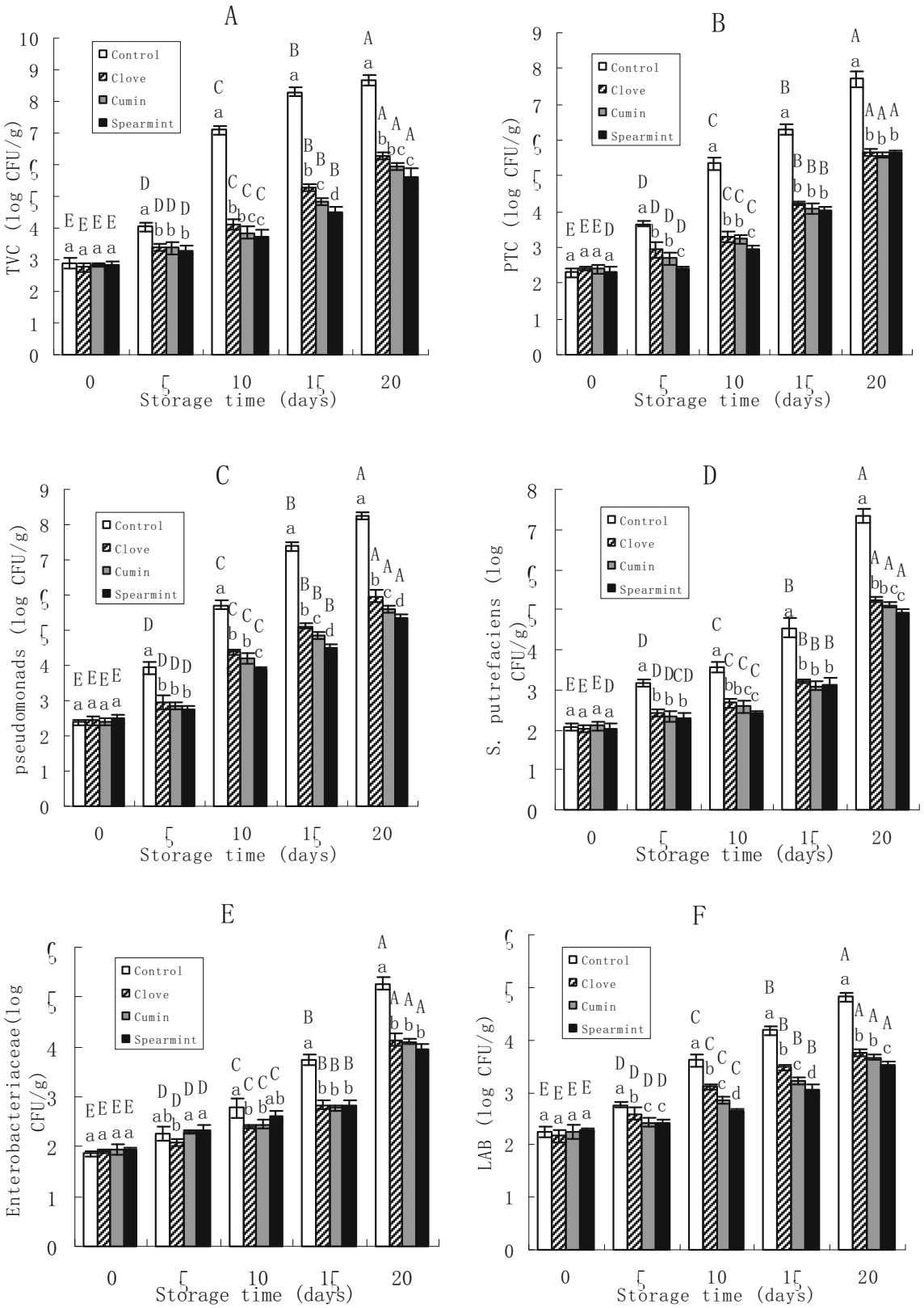


**Fig. 2** Changes in **a** total viable count, **b** psychrotrophic count, **c** pseudomonads, **d** *Shewanella putrefaciens*, **e** *Enterobacteriaceae* (), and **f** lactic acid bacteria count of turbot fillets stored at 2 °C for 20 days. Each data point is the mean of three replicate samples. *Vertical bars* represent standard deviation of means. Different *small letters* indicate significant differences ($p<0.05$) between treatments. Different *capital letters* indicate significant differences as storage time ($p<0.05$)

Similar results were reported by Jouki et al. (2014) who also found the successful inhibition of psychrotrophic bacteria growth in chilled rainbow trout fillets treated with oregano or thyme oils.

Nuin et al. (2008) reported pseudomonas as the primary specific spoilage organism in turbot degradation, with an important contribution to the TVC. It is noted that counts of pseudomonads were higher compared with those of other microbial classes due to a special cell membrane structure and the presence of cold-resistant compounds, resulting in partly resistant to low temperature (Bahmani et al. 2011). The initial population of *S. putrefaciens* was 2.08 log CFU/g, and on day 20 of storage, *S. putrefaciens* reached 7.36 log CFU/g in the control sample while in the presence of essential oils their counts were reduced by 5.25, 5.10, and 4.94 log CFU/g, respectively. The development of pseudomonas and *S. putrefaciens* led to the formation of off-odor during fish degradation, inhibited by essential oils with the high antimicrobial activities during storage (Pyrgotou et al. 2010). Enterobacteriaceae, as a part of the microflora of fresh turbot, were found to grow fast in the late stage of spoilage of turbot fillets. The samples treated with essential oils exhibited significant inhibitory effects on Enterobacteriaceae counts, which was consistent with the findings reported for different fish species, including common carp (Mahmoud et al. 2004) and rainbow trout (Pyrgotou et al. 2010; Alparslan et al. 2014). The LAB also constitutes substantial part of the natural microflora of fish, and in the present study, due to growing slowly at refrigerated condition, LAB counts increased but was low throughout the storage period.

## Conclusions

Successful control of quality in refrigerated turbot fillets was possible with essential oil fumigation treatments, such as clove, cumin, and spearmint. They exhibited lower microbial counts and better chemical quality. Moreover, essential oils fumigation not only possessed antimicrobial properties and retarded the lipid oxidation but also maintained texture and color characteristics. Results indicated that the shelf life of turbot fillets was 10 days for the control group and 20 days for the treated groups. It can be concluded that natural plant extracts can be used by the food industry to extend the shelf life because they exhibited promising antimicrobial and antioxidant effects.

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