

# The Effects of Essential Oils Addition on the Quality of Wild and Farmed Sea Bream (*Sparus aurata*) Stored in Ice

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**Abstract** The preservative effect of laurel (*Laurus nobilis*) and cumin (*Cuminum cyminum*) essential oils (EOs) on fresh vacuum-packed (VP) wild and farmed sea bream (*Sparus aurata*) fillets was evaluated during ice storage by microbiological, physicochemical and electrophoretic analyses. In the present study, wild (W) and farmed (F) fillet treatment included the following lots: control vacuum-packaged samples (WV and FV), VP with added EOs (0.5% v/w) of cumin (WVC and FVC), and of laurel (WVL and FVL). Wild and cultured fish were found to differ significantly in their muscle proximate compositions irrespective of fillet treatments with particularly higher fat and carbohydrate contents in farmed sea bream (4.82 and 0.32 g/100 g, respectively, vs. 1.53 and 0.22 g/100 g in wild fish). The treatment of wild and farmed sea bream fillets with laurel or with cumin EOs induced a decrease in bacterial growth by ca. 0.5 to 1 log cfu/g and in lipid oxidation by ca. 40% of TBA value, extending the shelf life of fish fillets by approximately 5 days of ice storage. However, the addition of EOs to VP fillets resulted in a reduced liquid holding capacity (LHC) throughout ice storage suggesting an early proteolysis initiation confirmed by the myofibrillar and sarcoplasmic electrophoretic profiles. Laurel and cumin EOs as natural and efficient antibacterial and antioxidant compounds can be used in

conjunction with VP to enhance ice-stored sea bream quality.

**Keywords** Sea bream · Essential oil · Laurel · Cumin · Vacuum-packaging · Quality · Liquid holding · Protein electrophoresis

## Introduction

Wild and farmed fish are gaining an increased importance as healthy food, because numerous species have been identified as rich in therapeutically important polyunsaturated fatty acids, easily digestible proteins, in addition to vitamins, and various other nutrients. Concurrently, important changes in pattern of food consumption are occurring including preference for safe and minimally processed foodstuffs. In the case of fish and seafood, food market analysts have estimated that changes will concern mainly changes in commodities; the overall tendency of seafood supply will show over the next decades, an increase of filleted and prepared/preserved fish and shellfish (Failler 2007). Changes in food consumption also included the preference of lightly preserved food products with natural additives (Burt 2004; Campos et al. 2010). For instance, spices and herbs extracts including their essential oils are becoming the emerging ingredients to fulfil part of the increasing demand for natural products with a “green image.”

Essential oils (EOs), obtained from plant material including flowers, seeds, leaves, herbs, fruits and roots, are mainly used in cosmetic and pharmaceutical and in food

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flavours as a number of EOs have been registered for use as flavourings in foodstuffs (Burt 2004). More recently, studies have shown the potential applications of EOs, solely or in combination with other preservation processes to conserve food (Mahmoud et al. 2006; Solomakos et al. 2008; Campos et al. 2010; Erkan 2010), and a few food preservatives containing EOs are already commercially available.

It is well known that EOs possess antibacterial, antioxidant, antiviral and antimycotic properties as reviewed by several authors (Burt 2004; Fisher and Phillips 2008). In cumin (*Cuminum cyminum*) and laurel (*Laurus nobilis*) EOs, such activities were assigned to their phenolic and terpenic compounds including cuminaldehyde,  $\beta$ -pinene,  $\rho$ -cymene, limonene, geranyl acetate, eugenol,  $\alpha$ -pinene and sabinene (Burt 2004; Gachkar et al. 2007), and  $\alpha$ -pinene, sabinene,  $\beta$ -pinene, 1,8-cineole, linalool, terpinen-4-ol,  $\alpha$ -terpineol and  $\alpha$ -terpinyl acetate (Bouzouita et al. 2001; Burt 2004), respectively. The biological properties of cumin EOs were also attributed to other substances such as cuminal and cuminic alcohol (Oroojalian et al. 2010). In most cases, the preservative effects have been extensively studied using a direct contact of antimicrobial assays, including different types of diffusion or dilution methods (Burt 2004; Mejhlholm, and Dalgaard 2002). The direct applications of cumin/laurel EOs and investigation effects on fish fillets are rather scarce. For instance, these EOs were found to extend the shelf life of mullet fish (Abou-Taleb et al. 2007), chub mackerel (Erkan and Bilen 2010), and bluefish (Erkan et al. 2010), without adversely affecting the sensory proprieties of fish fillets when used at relatively low concentration (1%).

The production of wild and cultured sea bream *Sparus aurata* in Mediterranean countries is playing a significant role in satisfying the increased demand for human consumption. However, there appears to be a stronger consumer preference for wild caught over cultured fish (Grigorakis 2007). It is therefore of great interest to reinforce the acceptance of cultured fish, and to increase its appeal by conducting comparative study on quality features and changes occurring in wild and cultured fish during post-harvesting procedures.

To our knowledge, there are no studies in the literature on wild/farmed sea bream treated with cumin or laurel EOs. Therefore, the objectives of the present work were to determine the effect of the treatment with cumin or laurel EOs, as a natural preservative, on quality features of fresh *S. aurata* stored in ice and to compare this effect on wild/farmed specimens by assessing some physicochemical and microbiological parameters. The effect of EOs on some technological aspects was equally included by investigating parameters such as muscle liquid holding and protein properties.

## Materials and Methods

### Fish Raw Materials

Fresh cultured gilthead sea bream (*S. aurata*; 45 fishes with average weight and length of  $192\pm 21$  g and  $235\pm 17$  mm, respectively) were obtained in March 2007 from the farming unit “SCALA Pêche Export,” located in Monastir (Tunisia). A culture land-based system and farm-made feeds (in the form of semi-moist pellets) were used for fish farming. Fish feed was prepared with fresh minced sardine (60%), fishmeal (20%), soybean oil cake (15%), wheat bran (3%), cod liver oil (0.5%), vitamin premix (1%) and mineral premix (0.5%) ingredients. Fish feed contained 55% protein, 19% fat, 13.5% carbohydrates, 11% ashes and 1.5% crud fibre as measured in dry matter. Fresh wild sea bream (45 fishes with average weight and length of  $169\pm 32$  g and  $254\pm 25$  mm, respectively) were caught by commercial longline fishing gear during the same day, from the coastal water of the same area (Monastir, Tunisia).

### Essential Oil Extraction

Dried cumin (*C. cyminum*) seeds and laurel (or sweet bay; *L. nobilis*) leaves were purchased from a local retail spice market (Tunis, Tunisia). EO was prepared by hydro-distillation of dried ground plant material (500 g of each) for 3 h using a Clevenger-type apparatus. The obtained EOs from each spice were dried separately using anhydrous sodium sulphate and stored in dark at 4 °C until required (Skandamis et al. 2000; Oroojalian et al. 2010).

### Preparation of Fish Samples and Storage Conditions

All fishes (wild/farmed) were killed by immersing in ice-cold water and delivered (packed into an insulated polystyrene box with ice) to the laboratory, within 3 h of harvesting. Upon arrival, fishes were immediately weighed, gutted, headed, washed and filleted. Six farmed (F) and wild (W) fish fillets were immediately sampled (day 0), two portions of tissues from each fillet were cut, one portion was served to microbiological analysis and the other deep immersed in liquid nitrogen and stored at  $-80$  °C until proximate/biochemical analysis. Each EO was added to wild/farmed filleted samples in appropriate volume (0.5% v/w) to the surface (two sides) of each fillet using a pipette followed by mild uniform distribution (directly with gloved fingers) of the oil for each sample. All filleted samples were vacuum-packaged at a local fish processing company (HDPM, Tunis, Tunisia) in food grade (EU Directive 2002/72/EC-2004/19/EC) polyethylene/polyamide pouches (16×25 cm, 100  $\mu$ m in thickness) provided by the same company, using a Multivac C550 packaging machine

(Multivac, Wolfertschwenden, Germany). Packed fillets (three fillets per pouch) were separated into six lots (30 fillets in each lot): wild and farmed fish fillets vacuum-packaged without EOs (WV and FV, respectively); wild and farmed fish fillets vacuum-packaged after addition of 0.5% cumin oil (WVC and FVC, respectively); and wild and farmed fish fillets vacuum-packaged after addition of 0.5% laurel oil (WVL and FVL, respectively). All packed fillets were immediately stored in ice (ratio of 1:1 (*w/w*)) into polystyrene boxes provided with holes for drainage. Boxes were covered and stored in a refrigerator (2–4 °C) for up to 20 days. Flesh sampling from each lot was performed on days 0, 5, 10, 15 and 20. All chemicals used in this work were obtained from Sigma-Aldrich-Fluka Company Ltd. (Poole, Dorset, UK), unless otherwise specified.

### Chemical Analysis

Crude fat was extracted using chloroform/methanol (2:1 *v/v*) solution according to Bligh and Dyer (1959). Total carbohydrates was analysed by using the colorimetric method of Dubois et al. (1956) with glucose as standard solution. Moisture content was determined by air drying of a portion of minced fish fillet at 103±2 °C for 24 h (method 950.46), crude protein analysis according to the Kjeldahl method using potassium sulphate and copper (II) sulphate as the catalysts (method 981.10), and ash by incineration in a muffle furnace at 550±2 °C for 24 h (method 938.08), according to the official methods of AOAC (1995).

### Microbiological Analysis

Sea bream flesh (10 g) obtained from each fillet were transferred aseptically to a Stomacher bag (Seward, West Sussex, UK) containing 90 ml of sterile 0.1% peptone water (Oxoid, Basingstoke, UK) and homogenised for 1 min using a laboratory blender Stomacher 400 (Seward, West Sussex, UK) at high speed. Tenfold successive dilutions were made with 0.1% peptone water from these homogenates as required. Bacterial counts were determined using plate count agar medium (Oxoid, Basingstoke, UK). The inoculated plates were incubated at 30 °C for 48 h and at 4 °C for 72 h for total viable mesophilic and psychrotrophic bacterial counts, respectively (Harrigan and McCance 1976). Microbiological counts were expressed as log colony-forming units (cfu) per gram of sample.

### TVB-N, TMA-N and Total Free Amino Acids (Ninhydrin-Positive Substances) Contents

Nitrogenous compounds were extracted from fish flesh as described in Attouchi and Sadok (2010). Analysis were

performed according to Ruiz-Capillas and Horner (1999) for total volatile basic nitrogen (TVB-N), to Sadok et al. (1996) for trimethylamine (TMA-N) and to Sadok et al. (1995) for total free amino acids measured as ninhydrin-positive substances (NPS).

### Thiobarbituric Acid Value

Thiobarbituric acid (TBA) value was measured directly on homogenised flesh sample according to the method of Hamre et al. (2001). TBA values were quantified by reference to malondialdehyde (MDA) as standard solution prepared from 1,1- to 3,3-tetraethoxypropan. Absorption was measured at 532 nm wavelength with a Smart Spec-plus spectrophotometer (Bio-Rad, Hercules, CA), and data were expressed as milligram MDA per kilogram sample.

### Determination of pH

Value of pH was determined, at room temperature, on homogenised fillet samples in distilled water (1:2 *w/v*) using a calibrated digital pH-meter (Inolab pH-720, WTW, Weilheim, Germany) according to AOAC process (method 981.12, AOAC 1995).

### Liquid-Holding Capacity Measurement

A centrifugation method, as described by Rørå et al. (2003), was applied to evaluate the liquid-holding capacity (LHC) of sea bream fillets. A portion of frozen chopped muscle samples (2.0 g) was placed in a centrifuge tube with a weighted (V1) filter paper (Whatman, Maidstone, England). Following muscle thawing, tubes were centrifuged (3K30 Sigma Centrifuge, Osterode am Harz, Germany) at 4,000×*g* for 10 min at 10 °C, and the wet paper was weighted (V2) before drying at 50 °C to constant weight (V3). The percentage value of liquid loss (LL) was calculated as  $100 \times (V2 - V1) / S$ , where *S*=weight of muscle sample, water loss as  $100 \times (V2 - V3) / S$  and fat loss as  $100 \times (V3 - V1) / S$ . All losses were expressed as percentage of muscle wet weight.

### Protein Extraction and Sodium Dodecyl Sulphate–Polyacrylamide Gel Electrophoresis

Muscle proteins were fractionated according to the procedure of Hashimoto et al. (1979). Samples were kept on ice throughout the process in order to prevent heating. Fish muscle (1 g) was homogenised in 10 ml of chilled phosphate buffer (15.6 mM Na<sub>2</sub>HPO<sub>4</sub>, 3.5 mM KH<sub>2</sub>PO<sub>4</sub>, pH 7.5) and then centrifuged at 5,000 *g* for 15 min at 4 °C. The pellet was re-suspended in 10 ml of the same buffer and centrifuged as mentioned above. Supernatants of both consecutive centrifugations were mixed together to constitute

the sarcoplasmic proteins fraction (SPP). Remaining pellets were again extracted twice with 10 ml of chilled phosphate buffer (15.6 mM Na<sub>2</sub>HPO<sub>4</sub>, 3.5 mM KH<sub>2</sub>PO<sub>4</sub>, 0.45 M KCl, pH 7.5) and centrifuged as previously described. Supernatants were collected as myofibrillar proteins fraction (MFP). The method of Bradford (1976) was used to quantify protein concentration in both fractions. The obtained SPP and MFP fractions were stored at -80 °C until analysis. Sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) of SPP and MFP fractions was performed according to Laemmli (1970) in a Mini Protean II electrophoresis system (Bio-Rad, Hercules, CA), using 5% polyacrylamide stacking gels and 15% separating gels. SPP and MFP extracts were diluted (4:1) in sample buffer (60 mM Tris–HCl, pH 6.8, 25% glycerol, 2% SDS, 0.1% bromophenol blue, 14.4 mM β-mercaptoethanol) and boiled for 5 min. Appropriate volumes were placed in each well in order to load ca. 30 and 35 μg proteins per well from SPP and MFP extracts, respectively. Protein molecular weight markers of 225, 150, 100, 75, 50, 35, 25 and 10 kDa (Promega, Madison, USA) were added (5 μl) to each gel. Electrophoresis was carried out at constant current of 30 mA per gel. Gels were then stained using Coomassie Brilliant Blue (R-250) method and analysed with a Digi-Doc-IT system (UVP, Upland, CA).

#### Statistical Analysis

All experiments were carried out in duplicate on different occasions. Analyses were run in triplicate for each replicate and averaged for each fillet. Results were presented as mean ± standard deviation. Data from the different measurements were subjected to ANOVA using SPSS version 11.01 software (SPSS Chicago, IL, USA). Tukey's test was used to check for significant differences among mean values at the 5% level. Data were also explored by principal component analysis (PCA; Martens and Næs 1989) using multivariate statistical software (The Unscrambler version 9.2, CAMO Software AS, Oslo, Norway). Leverage correction of all the data was applied. The variables were weighted with the inverse of the standard deviation of all the objects in order to compensate for the different scales of the variables.

## Results and Discussion

#### Proximate Composition

Proximate composition as a percentage of wet-weight fillets in fresh wild sea bream is given in Table 1. Results are similar to that reported in a previous work (Attouchi and Sadok 2010). Fat and carbohydrates contents were high ( $p <$

**Table 1** Proximate composition of fresh wild and farmed *S. aurata*

	Wild	Farmed
Moisture <sup>a</sup>	79.17±1.01a	75.10±1.03b
Protein <sup>b</sup>	19.04±1.02a	19.95±0.91a
Total lipid <sup>c</sup>	1.53±0.27a	4.82±0.44b
Ash <sup>d</sup>	1.36±0.10a	1.42±0.11a
Carbohydrate <sup>e</sup>	0.22±0.03a	0.32±0.03b

Data are mean ± standard deviation,  $n=6$ . Means within the same row with different superscript are significantly different ( $p < 0.05$ )

<sup>a–e</sup> Gram per 100 g wet fillet

0.05) in farmed sea bream, while wild fish showed a higher ( $p < 0.05$ ) moisture content. Such differences were well established in other studies (Flos et al. 2002; Grigorakis 2007), and have been attributed to the high dietary fat level in the feed and to reduced activity of cultured fish. Additionally, the unlimited access to feed in intensive sea bream farming system leads to increased muscle carbohydrates (Kristoffersen et al. 2006). In contrast, no significant differences ( $p > 0.05$ ) in protein and ash contents were found between wild/farmed flesh fish lots. Similar results have been also obtained in other works, and protein content variation was reported to be mainly determined by species type, genetic characteristics and fish size (Grigorakis 2007; Attouchi and Sadok 2010).

During ice storage, the moisture and fat contents showed no significant changes (Table 2) with mean values of 79.55% and 75.56% and 1.54% and 4.90% respectively for wild and cultured fish. Statistic analysis has shown that moisture and fat contents were not significantly influenced ( $p > 0.05$ ) by storage time nor by EOs treatment in all fish groups vacuum-packaged fillet. Similar results were found for wild/cultured sea bream when treated with powdered thyme (Attouchi and Sadok 2010).

#### Microbiological Changes

Changes in the microbial flora of all filleted fish samples during ice storage are illustrated in Table 3, representing a foremost comparative study for wild and cultured sea bream. Mesophilic bacterial counts (MBC) in fresh wild/farmed sea bream were respectively 3.22±0.53 and 2.83±0.26 log cfu/g, while psychrotrophic bacterial counts (PBC) were respectively 3.55±0.42 and 3.11±0.29 log cfu/g. Considering the proposed upper limit for aerobic plate count of  $5 \times 10^5$  cfu/g for fresh fish (ICMSF 1986), MBC and PBC showed relatively low initial values in all samples, indicating the good quality of fish and that fish processing was done accurately. Initial microbial counts in both fish populations were found to be similar to those reported in cultured sea bream by Chouliara et al. (2004). Furthermore,

**Table 2** Changes in the moisture and total fat of each sea bream fillet groups during storage period

Days	Analysis	Group WV	Group WVC	Group WVL	Group FV	Group FVC	Group FVL
0	Moisture <sup>a</sup>	79.17±1.01a	79.17±1.01a	79.17±1.01a	75.10±1.03b	75.10±1.03b	75.10±1.03b
	Total fat <sup>b</sup>	1.53±0.27a	1.53±0.27a	1.53±0.27a	4.82±0.44b	4.82±0.44b	4.82±0.44b
5	Moisture	79.71±0.74a	81.15±0.69a	79.93±1.81a	76.77±0.75a	74.93±1.34a	76.07±1.31a
	Total fat	1.68±0.35a	1.38±0.22a	1.43±0.21a	4.98±0.45a	4.95±0.55a	4.88±0.37a
10	Moisture	80.44±0.61a	77.45±0.48a	77.64±0.70a	75.35±1.31a	74.17±1.38a	73.53±1.64a
	Total fat	1.52±0.38a	1.43±0.36a	1.57±0.23a	4.83±0.59a	5.12±0.61a	4.72±0.68a
15	Moisture	80.60±0.48a	79.70±0.57a	78.04±0.69a	75.18±0.90a	75.11±0.91a	75.61±1.80a
	Total fat	1.67±0.22a	1.42±0.41a	1.78±0.43a	4.58±0.46a	4.82±0.68a	5.05±0.66a
20	Moisture	80.58±0.64a	81.09±0.74a	78.28±0.87a	76.57±1.73a	76.21±1.12a	77.16±1.13a
	Total fat	1.42±0.15a	1.57±0.19a	1.63±0.20a	5.38±0.60a	4.45±0.60a	4.98±0.39a

WV vacuum-packaged wild sea bream fillets, WVC vacuum-packaged wild sea bream fillets treated with 0.5% cumin EO, WVL vacuum-packaged wild sea bream fillets treated with 0.5% laurel EO, FV vacuum-packaged farmed sea bream fillets, FVC vacuum-packaged farmed sea bream fillets treated with 0.5% cumin EO, FVL vacuum-packaged farmed sea bream fillets treated with 0.5% laurel EO

Data are mean ± standard deviation,  $n=6$ . Means within the same row with different superscript are significantly different ( $p<0.05$ )

<sup>a-b</sup> Gram per 100 g wet fillet

no significant difference ( $p>0.05$ ) was found in initial MBC and PBC values between wild/farmed sea bream lots.

During iced storage, bacterial load growth in vacuum-packaged wild (WV) and farmed (FV) sea bream was characterised by a preliminary lag phase and showed a raise after 10 days of storage. Compared to WV group, PBC loads were lower ( $p<0.05$ ) at day 5 and become higher ( $p<0.05$ ) at day 20 of storage in FV group. In contrast, MBC values did not show any significant differences between WV and FV groups all over the storage period. During aerobic ice storage of cod, Herland et al. (2007) and Olsson et al. (2007) have found higher aerobic plat and specific

spoilage bacteria counts in wild than in farmed fillets. This distinction in microbial loads has been related to chemical differences of flesh between both cod groups. In this way, according to Calo-Mata et al. (2008) and Cortesi et al. (2009), the post-mortem microbial status of seafood is closely related to environmental conditions, water microbiological quality, the type of fishing and handling conditions. In particular, in intensive marine aquaculture, the kind/quality of feed and the rearing density can affect the microbial status of cultured fish. Treatment with cumin or laurel EOs showed a significant bacterial growth reduction ( $p\leq 0.05$ ) in all wild/farmed VP groups throughout storage

**Table 3** Changes in mesophilic (MBC) and psychrotrophic (PBC) bacteria counts in each sea bream fillet groups during the storage period

Days	Analysis	Group WV	Group WVC	Group WVL	Group FV	Group FVC	Group FVL
0	MBC <sup>a</sup>	3.22±0.53a	3.22±0.53a	3.22±0.53a	2.83±0.26a	2.83±0.26a	2.83±0.26a
	PBC <sup>b</sup>	3.55±0.42a	3.55±0.42a	3.55±0.42a	3.11±0.29a	3.11±0.29a	3.11±0.29a
5	MBC	3.36±0.66a	ND	2.42±0.24b	2.80±0.17ab	2.77±0.28ab	ND
	PBC	4.14±0.38a	ND	ND	3.29±0.30b	ND	2.35±0.15c
10	MBC	4.31±0.75ab	3.15±0.50ac	3.33±0.51ac	4.58±0.03b	4.24±0.06ab	2.65±0.16c
	PBC	4.97±0.12a	3.85±0.15bc	4.43±0.34ac	4.59±0.12a	3.62±0.38b	3.84±0.33bc
15	MBC	6.33±0.08ac	5.35±0.10b	5.77±0.24ab	6.80±0.31c	5.31±0.32b	5.20±0.36b
	PBC	6.42±0.20a	5.43±0.18b	5.49±0.02b	6.64±0.14a	5.27±0.36b	5.47±0.13b
20	MBC	6.73±0.19ab	6.59±0.11ab	6.72±0.02ab	7.24±0.03a	6.30±0.33b	6.38±0.54b
	PBC	6.72±0.23a	6.20±0.10b	6.30±0.16ab	7.62±0.14c	6.16±0.21b	6.13±0.20b

WV vacuum-packaged (VP) wild sea bream fillets, WVC VP wild sea bream fillets treated with 0.5% cumin EO, WVL VP wild sea bream fillets treated with 0.5% laurel EO, FV VP farmed sea bream fillets, FVC VP farmed sea bream fillets treated with 0.5% cumin EO, FVL VP farmed sea bream fillets treated with 0.5% laurel EO, ND not detected

Data are mean ± standard deviation,  $n=6$ . Means within the same row with different superscript are significantly different ( $p<0.05$ )

<sup>a-b</sup> Log cfu per gram

period. Particularly, at day 5 of storage, MBC were not detectable in WVC and FVL groups, as well as PBC that were not measurable in WVC, WVL and FVC groups at this time. This finding confirmed the bacteriostatic nature of these two EOs and their inhibitory effect on the development of the natural microflora. Comparable data were observed in cold-stored mullet fish fillets when treated with laurel/cumin ethanol extracts (Abou-Taleb et al. 2007). The antibacterial activity of cumin EO was assigned to its phenolic and terpenic compounds (Burt 2004; Gachkar et al. 2007), as well as its cuminal and cuminic alcohol compounds (Oroojalian et al. 2010). Antimicrobial activities of laurel EO were also attributed to its phenolic and terpenic components (Bouzouita et al. 2001; Burt 2004). In general, EOs antimicrobial mechanism of action was considered to be the disturbance of the cytoplasmic membrane, disruption of the proton-motive force, electron flow, active transport and coagulation of bacteria contents (Burt 2004).

MBC and PBC values exceeded the value of 7 log cfu/g (upper acceptability limit for freshwater and marine species; ICMSF 1986) on day ca. 16 in FV samples. Such value has never been reached in wild and EO-treated samples up to 20-day storage period. Chouliara et al. (2004) reported that mesophilic counts of salted VP farmed sea bream fillets reached the value of 7 log cfu/g after 14 days storage at 4 °C.

#### Changes in TVB-N and TMA-N

Development of TVB-N values in all sea bream fillet lots is given in Table 4. At the beginning and throughout ice

storage, WV lot had significantly ( $p < 0.05$ ) lower TVB-N contents than farmed fish. The same observation was noticed in aerobically ice-stored wild/farmed sea bream (Attouchi and Sadok 2010). Such difference may be explained by the higher level of non-protein nitrogenous compounds in farmed sea bream flesh (Kyra et al. 1997), which was related to several factors such as fish feeding type, catching season and fish size (Goulas and Kontominas 2007), as well as fish age, locality and culture method (Kyra et al. 1997). As noted in Table 4, TVB-N levels revealed a significant raise with different rates in all fillet batches during storage period. The TVB-N level increases followed the order WVL-WVC < FVC < FVL < WV < FV. TVB-N values exceeded the upper acceptability limit (35 mg N/100 g of fish flesh) set by the European commission (CEC 1995) on day ca. 16 and 20 in FV and WV groups, respectively, while all other batches did not reach this limit until the end of storage period. Thus, for all fillet samples, a relative correlation can be noted between the point of their microbiological rejection (7 log cfu/g) and the point of their biochemical rejection based on TVB-N value (35 mg N/100 g). These results indicate that TVB-N can be used as a spoilage index for sea bream fillets in the present experiment. Cumin or laurel EO treatment combined with VP produced an obvious preservative effect lowering significantly ( $p < 0.05$ ) the TVB-N levels of all EO-treated fish fillets. The present data concurred with those obtained by the application of laurel/cumin ethanol extracts in cold-stored mullet fish (Abou-Taleb et al. 2007). Indeed, this finding may be attributed to the inhibition of spoilage bacteria activity caused by the antibacterial properties of active compounds of these EOs as explained

**Table 4** TVB-N and TMA-N changes of each fillet groups during the storage period

Days	Analysis	Group WV	Group WVC	Group WVL	Group FV	Group FVC	Group FVL
0	TVB-N <sup>a</sup>	11.07±0.85a	11.07±0.85a	11.07±0.85a	13.20±0.74b	13.20±0.74b	13.20±0.74b
	TMA-N <sup>b</sup>	0.23±0.05a	0.23±0.05a	0.23±0.05a	0.17±0.03a	0.17±0.03a	0.17±0.03a
5	TVB-N	13.87±2.20a	13.49±0.65a	13.49±1.91a	19.90±0.68b	16.95±0.70c	17.28±1.46c
	TMA-N	0.16±0.02a	0.75±0.04b	0.49±0.04c	0.13±0.02a	0.42±0.05d	0.43±0.02d
10	TVB-N	14.14±2.44ac	14.15±1.62ac	12.67±0.59a	21.42±1.73b	19.06±0.38b	16.08±0.81c
	TMA-N	0.25±0.01a	0.92±0.06b	0.54±0.02cd	0.68±0.12e	0.59±0.04ce	0.44±0.02d
15	TVB-N	24.59±1.26a	15.63±0.90b	15.14±1.77b	33.77±2.01c	21.04±0.89a	20.02±2.12a
	TMA-N	1.81±0.17a	1.09±0.07b	0.63±0.04c	2.96±0.19d	1.26±0.10b	0.84±0.08e
20	TVB-N	35.80±1.59a	19.87±0.66b	18.74±1.47b	41.43±2.06c	27.67±0.52d	32.01±1.39e
	TMA-N	4.42±0.29a	1.06±0.10be	0.75±0.03b	3.25±0.20c	2.77±0.24d	1.22±0.09e

WV vacuum-packaged (VP) wild sea bream fillets, WVC VP wild sea bream fillets treated with 0.5% cumin EO, WVL VP wild sea bream fillets treated with 0.5% laurel EO, FV VP farmed sea bream fillets, FVC VP farmed sea bream fillets treated with 0.5% cumin EO, FVL VP farmed sea bream fillets treated with 0.5% laurel EO

Data are mean ± standard deviation,  $n=6$ . Means within the same row with different superscript are significantly different ( $p < 0.05$ )

<sup>a-b</sup> Milligram N per 100 g

above. In consequence, the use of cumin or laurel EOs combined with VP extended the shelf life of fish fillets by about 5 days in both wild/farmed sea bream.

It is worth noting that the preservative effect of cumin or laurel EOs was more prominent in wild than in farmed fish samples (Table 4). Such results may be explained by the diluting effect of the oily matrix of fish on the EO phenolic/terpenic lipophilic components reducing their antibacterial effectiveness as has been reported by Burt (2004).

Changes in TMA-N values in all samples during ice storage are exposed in Table 4. At day 0, low TMA-N contents with no significant ( $p>0.05$ ) difference were recorded in fresh wild/farmed sea bream fillets, indicating good freshness of the product. These values were in the range of TMA-N levels noted in fresh sea bream in other studies (Goulas and Kontominas 2007; Attouchi and Sadok 2010). Throughout the refrigerated storage, TMA-N levels increased exponentially with  $r^2>0.7$  in all fish fillets. The TMA contents rose in the order  $WVL<WVC<FVL<FVC<FV<WV$ . After 20 days of storage, a higher significant TMA-N level was recorded in WV group than in FV group. This difference can be attributed to the initial difference in TMAO content between wild/farmed fish, as reported by Herland et al. (2007). Wide range of TMA-N values has been reported to set the acceptability limit: 1 mg N/100 g (Kyrana et al. 1997); 2–3 mg N/100 g (Goulas and Kontominas 2007); and 10–15 mg N/100 g (Connell 1990). Taking into account the microbiological and TVB-N results, a value of 2–3 mg N/100 g was more realistic to set TMA-N limit of acceptability in the present storage conditions. Assuming the TMA-N value of 3 mg N/100 g as acceptability threshold, farmed (FV) and wild (WV) sea bream samples

exceeded this value after ca. 15 and 18 days, respectively, such values were not reached in EO-treated samples throughout the entire storage period. Thus, the preservative action of cumin or laurel EOs treatment can be established and may be attributed to antibacterial properties of active compounds of the considered EOs as previously described. This finding matched also those acquired by Abou-Taleb et al. (2007). As shown in Table 4, the effect of cumin or laurel EOs was more accentuated in wild than in farmed fish fillets; this fact may be explained by the dilution action of high fat content in farmed fish on active compounds of EOs (Burt 2004).

#### Changes in Free Amino Acids (NPS) and TBA

Free amino acids are produced in fish as a result of muscle proteolysis, and as muscle spoilage progresses, these compounds serve as a substrate for microbial growth (Ruiz-Capillas and Moral 2003). NPS values recorded during chilled storage of all sea bream samples are indicated in Table 5. Initially, mean NPS level of fresh farmed sea bream fillets was slightly higher than that of wild fish with no significant difference ( $p>0.05$ ).

Throughout ice storage, NPS values of WV and FV groups remained constant throughout storage, and showed significant increase at the end of this period. Ruiz-Capillas and Moral (2003) reported that levels of free amino acids in hake, packed under combined system of atmospheres, fluctuated or remained constant all over chilled storage. In the present study, EO-treated lots had undergone a slight increase in NPS levels at day 5 and some fluctuations all over this period with no significant differences. The earlier

**Table 5** NPS and TBA changes of each fillet groups during the storage period

Days	Analysis	Group WV	Group WVC	Group WVL	Group FV	Group FVC	Group FVL
0	NPS <sup>a</sup>	0.93±0.38a	0.93±0.38a	0.93±0.38a	1.24±0.37a	1.24±0.37a	1.24±0.37a
	TBA <sup>b</sup>	0.25±0.02a	0.25±0.02a	0.25±0.02a	0.41±0.07b	0.41±0.07b	0.41±0.07b
5	NPS	0.93±0.29a	1.63±0.40b	1.36±0.36ab	1.12±0.27ab	1.51±0.38ab	1.55±0.35ab
	TBA	0.56±0.11ac	0.42±0.04ab	0.28±0.05b	0.62±0.14c	0.45±0.09a	0.48±0.07ac
10	NPS	0.89±0.27a	1.13±0.33ab	0.96±0.19ab	1.02±0.28ab	1.43±0.31b	1.17±0.15ab
	TBA	0.84±0.09a	0.77±0.07a	0.21±0.02b	0.71±0.07ac	0.59±0.12c	0.61±0.03c
15	NPS	0.98±0.16a	0.88±0.20a	0.90±0.30a	1.13±0.43a	1.31±0.34a	1.25±0.26a
	TBA	0.91±0.06a	0.71±0.09be	0.36±0.04c	1.05±0.09d	0.80±0.06ab	0.63±0.05e
20	NPS	1.55±0.41ab	1.46±0.44ab	1.01±0.30a	1.87±0.24b	1.25±0.26ab	1.14±0.37a
	TBA	1.39±0.09a	0.89±0.10b	0.81±0.12b	1.48±0.12a	0.93±0.08b	0.85±0.11b

WV vacuum-packaged (VP) wild sea bream fillets, WVC VP wild sea bream fillets treated with 0.5% cumin EO, WVL VP wild sea bream fillets treated with 0.5% laurel EO, FV VP farmed sea bream fillets, FVC VP farmed sea bream fillets treated with 0.5% cumin EO, FVL VP farmed sea bream fillets treated with 0.5% laurel EO

Data are mean ± standard deviation,  $n=6$ . Means within the same row with different superscript are significantly different ( $p<0.05$ )

<sup>a</sup> Millimole AA per 100 g

<sup>b</sup> Milligram MDA per kilogram

rise of NPS in WVC, WVL, FVC and FVL samples concurred with their lowest microbiological counts, while the following fluctuations reflected the resumed bacteria growth as muscle spoilage progresses.

Assessment of lipid oxidation was undertaken by TBA measurement in all VP fillets lots during storage (Table 5). Low initial TBA content was observed in fresh farmed/wild sea bream (0.41 and 0.25 mg MDA/kg, respectively) with a significant difference ( $p < 0.05$ ) between the two fish types. Throughout the chilled storage, a slow rate increase ( $p < 0.05$ ) was noted in TBA levels of WV as compared to FV group. This finding was also recorded in a previous work as a result of lower fat content in wild fish and higher level of n-3 polyunsaturated fatty acids in farmed fish (Grigorakis 2007). While referring to previous TBA values obtained in aerobically ice-stored wild/farmed sea bream (Attouchi and Sadok 2010), the use of VP alone or in combination with cumin or laurel EOs played a key role in keeping TBA values at low levels in all fillet samples throughout the entire storage period. Moreover, TBA content of laurel treated under VP sample (particularly WVL) was extremely low. In literature, it has been stated that use of the whole cumin seeds and laurel leaves or their extracts, possessing

strong antioxidant activity, (Burt 2004; Gachkar et al. 2007) can control lipid oxidation in muscle food such as mullet fish (Abou-Taleb et al. 2007) and frozen chub mackerel (Erkan and Bilen 2010). The antioxidant activity of both cumin and laurel EOs was related to their phenolic, terpenic and other compounds as cited above. According to Connell (1990), TBA levels of 1–2 mg MDA/kg of fish flesh are generally considered as the limit beyond which fish will normally develop off-flavours and off-odours. The TBA contents of the present sea bream fillet samples exceeded the value of 1 mg MDA/kg after ca. 15 and 18 days of storage period for (FV) and (WV) groups, respectively, while WVC, WVL, FVC and FVL groups never reached this limit value throughout the whole storage period.

#### Changes in pH, LHC, WHC and Fat Loss

Assessment of pH values in all fish samples during ice storage is displayed in Table 6. A significant difference ( $p < 0.05$ ) was noted in starting values of pH between fresh wild/farmed sea bream ( $6.65 \pm 0.03$  and  $6.22 \pm 0.02$ , respectively). The same difference were sighted in wild/cultured fish species such

**Table 6** Liquid loss (LL), water loss (WL), fat loss (FL) and pH changes of each sea bream fillet groups during the storage period

Days	Analysis	Group WV	Group WVC	Group WVL	Group FV	Group FVC	Group FVL
0	pH	6.65±0.03a	6.65±0.03a	6.65±0.03a	6.22±0.02b	6.22±0.02b	6.22±0.02b
	LL <sup>a</sup>	15.07±1.19a	15.07±1.19a	15.07±1.19a	18.75±0.71b	18.75±0.71b	18.75±0.71b
	WL <sup>b</sup>	13.69±0.95a	13.69±0.95a	13.69±0.95a	15.89±0.73b	15.89±0.73b	15.89±0.73b
	FL <sup>c</sup>	1.38±0.38a	1.38±0.38a	1.38±0.38a	2.86±0.28b	2.86±0.28b	2.86±0.28b
5	pH	6.74±0.02a	6.66±0.01b	6.58±0.01c	6.28±0.02d	6.41±0.01e	6.30±0.01d
	LL	16.15±0.95a	24.21±1.54b	22.60±0.73bc	20.32±1.73c	23.16±0.88b	24.31±1.76b
	WL	14.77±0.79a	22.65±1.41b	21.17±0.73bd	17.66±1.54c	19.28±0.81cd	20.60±1.70bd
	FL	1.38±0.17a	1.56±0.18b	1.43±0.10a	2.65±0.29b	3.88±0.60c	3.71±0.43c
10	pH	6.65±0.02a	6.62±0.01b	6.61±0.02b	6.42±0.02c	6.43±0.01c	6.26±0.01d
	LL	16.33±1.15a	23.56±1.61bd	23.52±1.26bd	19.25±0.93c	24.25±1.49b	21.74±1.58d
	WL	14.97±0.93a	21.98±1.44bc	22.09±1.12b	16.43±0.78ad	19.84±1.54ce	18.20±1.49de
	FL	1.36±0.33a	1.58±0.35a	1.43±0.32a	2.82±0.35b	4.41±0.48c	3.54±0.27d
15	pH	6.71±0.02a	6.64±0.01b	6.68±0.03c	6.33±0.02d	6.44±0.01e	6.31±0.01d
	LL	19.54±1.93a	25.09±1.36b	24.14±1.06b	19.74±1.95a	25.67±1.44b	24.23±1.46b
	WL	18.22±1.76ad	23.53±1.33b	22.71±0.93bc	16.67±1.65a	21.96±1.29bc	20.77±1.68cd
	FL	1.32±0.19a	1.56±0.23a	1.44±0.23a	3.07±0.40b	3.71±0.40c	3.46±0.31bc
20	pH	6.94±0.02a	6.67±0.01b	6.63±0.01c	6.36±0.01d	6.53±0.01e	6.36±0.02d
	LL	19.76±1.71a	27.28±1.30b	23.46±1.42c	22.22±0.81ac	27.25±1.32b	23.87±1.95c
	WL	18.43±1.62a	25.81±1.12b	22.18±0.97cd	18.70±0.88a	23.29±1.29c	20.54±1.94ad
	FL	1.34±0.12a	1.47±0.22a	1.28±0.56a	3.52±0.46b	3.96±0.37b	3.33±0.62b

WV vacuum-packaged (VP) wild sea bream fillets, WVC VP wild sea bream fillets treated with 0.5% cumin EO, WVL VP wild sea bream fillets treated with 0.5% laurel EO, FV VP farmed sea bream fillets, FVC VP farmed sea bream fillets treated with 0.5% cumin EO, FVL VP farmed sea bream fillets treated with 0.5% laurel EO

Data are mean ± standard deviation,  $n=6$ . Means within the same row with different superscript are significantly different ( $p < 0.05$ )

<sup>a-c</sup> Gram per 100 g wet fillet



as cod (Kristoffersen et al. 2006; Herland et al. 2007; Olsson et al. 2007), Atlantic halibut (Olsson et al. 2003a; b) and sea bream (Flos et al. 2002; Attouchi and Sadok 2010). The low ultimate pH in farmed fish was generally reported to be due to the higher glycogen content prior to slaughtering. Thus, in intensive fish farming, unlimited access to feed leads to increase muscle glycogen and subsequently to reduce muscle post-mortem pH in response to anaerobic degradation of glycogen (Kristoffersen et al. 2006). Over the storage period, pH value had undergone different change patterns in sea bream batches. In WV lot, pH showed a significant rise towards the end of storage time, whereas pH values of WVC and WVL lots remained unaffected during this period. As reported in literature, the increase of pH values during storage was attributed to the production of basic compounds such as ammonia, trimethylamine and other biogenic amines by fish spoilage bacteria (Kyrana et al. 1997). In this study, pH values of wild sea bream fillets (WV, WVC and WVL) demonstrated a good correlation with TVB-N and TMA-N changes. In addition, pH stability observed in WVC and WVL samples may be due to EO inhibitory effects on microbial growth, which in turn, delay the formation of basic nitrogen compounds.

In FV lot, pH values showed a significant increase only after 10 days of storage which coincided with microbiological loads rise, followed by a decrease towards the end of this period. This observation may be related to the growth of lactic acid bacteria (LAB) as suggested by numerous reports. Indeed, LAB bacteria are facultative anaerobic species, naturally present in seafood (Calo-Mata et al. 2008) and in relatively large number in vacuum-packaged farmed sea bream (Chouliara et al. 2004) as well as in farmed cod (Olsson et al. 2007). Such bacteria may contribute to unchanged or declined muscle pH, primarily by producing lactic acid which acts to buffer basic metabolites, and secondly by producing bacteriocin which acts to inhibit growth of other kind of bacteria (Herland et al. 2007; Calo-Mata et al. 2008). As shown in Table 6, pH values had undergone a preliminary increase at day 5 in FVC group and remained unchanged towards the end of storage period, which was the case of FVL lot all over this period. Furthermore, it is worth noting that despite its relatively higher TVB-N and TMA-N levels (see Table 4), final pH values recorded in FVC and FVL lot were significantly ( $p < 0.05$ ) less pronounced than those of treated wild fish. Such data may be also associated with lactic LAB activity in farmed fillets, which were reported to have high tolerance towards the action of EOs due to their ability to generate ATP and to deal with conditions of osmotic stress (Burt 2004).

It is well known that determination of LHC in meat and fish is important for economical reasons (weight decrease due to water loss) and for sensory properties (colour,

juiciness and tenderness; Olsson et al. 2003a). Variations of fat loss (FL), water loss (WL) and liquid loss (LL) levels in different treatments are included in Table 6. Initially and throughout ice storage, the FL in wild fillets were significantly ( $p < 0.05$ ) lower than in farmed fish independently of EOs treatment. Such result confirmed results found in an earlier study (Attouchi and Sadok 2010). During storage, EOs treatment showed no net effect on FL levels in all fish fillets. The dissimilarity of FL levels between wild/farmed fish could be explained by the difference in fat and in collagen contents between both sea bream groups (Rørå et al. 2003; Attouchi and Sadok 2010).

In the present experiment, high LL and WL starting values ( $p < 0.05$ ) were recorded in farmed sea bream fillets as compared with those of wild fish, allocating to the latter a better liquid-binding properties (Table 6). In literature, the same observations were perceived in wild/farmed sea bream (Flos et al. 2002; Attouchi and Sadok 2010) and in other wild/cultured species such as Atlantic halibut (Olsson et al. 2003a; b) and cod (Olsson et al. 2007). The elevated LL in farmed fish was attributed to its low initial pH values (Olsson et al. 2003b). In aquaculture system, intensive feeding of fish leads to particularly low ultimate pH, affecting the volume of the myofibrils in muscle, which has been shown to lower its LHC (Olsson et al. 2003b; 2007). Always, as illustrated in Table 6, time storage affected LHC levels of both wild/farmed samples, and additionally, it was mostly clear that WL changes were more correlated to LL in all batches; hence, the sea bream LL variation throughout the ice storage seemed to be more related to water loss than to fat loss. The LL levels revealed a slight but significant increase ( $p < 0.05$ ) after 15 and 20 days of ice storage in WV and FV lots, respectively, whereas in an earlier study (Attouchi and Sadok 2010), LL values of wild/farmed sea bream fillets increased significantly after 7 days of aerobic ice storage, suggesting that application of vacuum-packaging may delay LL changes in fish fillets.

However, when fish fillets were treated with EOs, significant LL increase ( $p < 0.05$ ) was observed in WVC, WVL, FVC and FVL after 5 days of chilled storage (Table 6). Furthermore, the nature of the EOs seems to have an effect on LL changes. Thus, LL values showed a continuous increase ( $p < 0.05$ ) during storage period in cumin EO-treated lots, whereas it remained unchanged in laurel EO-treated lots. The scarcity of data on the combined effect of EOs and vacuum packaging limited result interpretation. However, Goulas and Kontominas (2007) reported that changes in LHC was more related to decrease in pH values caused by  $\text{CO}_2$  dissolution in modified atmosphere packaging of sea bream fillets treated with oregano EOs.

In this study, data in Table 6 suggest that other factors than pH are also associated to LHC variation. Numerous and complex factors were reported to contribute to the LHC weakness, such as ionic strength, pH, temperature (Olsson et al. 2003b), and change in the muscle fibres configuration caused by endogenous enzymes activities (Olsson et al. 2003a). Thus, it was suggested that enzymes including cathepsins and calpains could potentially influence muscle LHC, as the majority of the water in the flesh is bound to myofibrils (Hagen et al. 2008).

Accordingly, in a reducing environment, as is the case of vacuum packaging, endogenous calpain enzymes may be activated inducing the early increase in LL levels as was suggested by Huff-Lonergan and Lonergan (2005). Furthermore, as reported by Huff-Lonergan and Lonergan (2005), high levels of antioxidants in meat can improve proteolysis. Consequently, it is convenient to assume that the presence of antioxidant substance such as EOs, combined with reducing atmosphere (vacuum-packaging), would influence calpain activity, proteolysis, and hereby quality characteristics such as LHC. Moreover, growth of some specific spoilage bacteria and interaction between them can be implicated in fish muscle-LHC variation (Olsson et al. 2007).

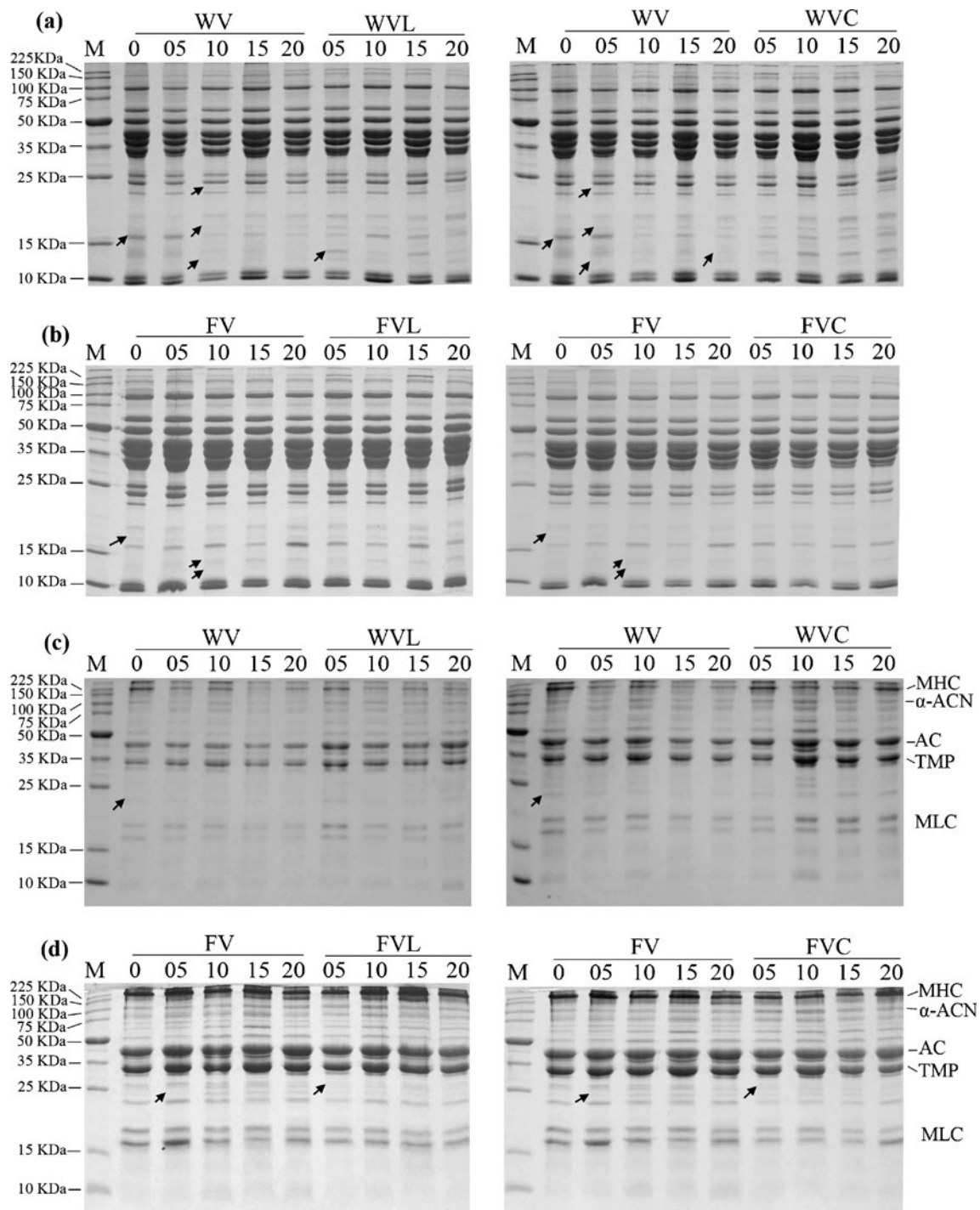
#### Electrophoretic (SDS–PAGE) Analysis

Changes in electrophoretic profiles of SPP and MFP extracts of all sea bream treatments are shown in Fig. 1. SPP extracts of wild and farmed fillets lots (Fig. 1a, b, respectively) contained 11 principal bands after SDS–PAGE separation, with relative molecular weight of 100, 62, 50, 41, 36, 33, 26, 24, 22, 10 and 9 kDa. Several fractions were obtained for MFP extracts of wild and farmed fillets lots (Fig. 1c, d, respectively), although the most contributing to total protein content, in terms of optical density, were the bands of 200, 108, 42, 32, 18 and 16 kDa. According to Delbarre-Ladrat et al. (2006) and several other studies, these bands were tentatively identified as myosin heavy chain (MHC),  $\alpha$ -actinin ( $\alpha$ -ACN), actin (AC), tropomyosin (TMP) and myosin light chains (MLC), respectively. At first sight, comparison of SPP and MFP electrophoretic patterns among fresh wild/farmed fish did not show evident differences between the two fish types. In general terms, no substantial changes were observed in the SSP and MFP extract profiles of all sea bream lots as a consequence of treatments and of storage progression. However, minor changes were mainly noted in low molecular weight protein bands in both fractions. For instance, electrophoregram of SSP extract showed the appearance of 23, 18 and 12 kDa and the decrease of 16-kDa band density from the day 10 of ice storage in WV group, whereas these polypeptides were revealed in

addition to a 14-kDa band from the day 5 of storage period in WVC and WV groups (Fig. 1a). In FV lot, SSP electrophoresis profile showed the appearance of 14- and 12-kDa bands from the day 10 of ice storage, and the increase of 18-kDa band density at the end of this period, while these bands were observed from the day 5 of storage period in FVC and FVL groups with different densities (Fig. 1b). MFP electrophoretic pattern indicated the occurrence of low density 22 kDa band in WV and WV groups from the day 0 and 5, respectively, the concentration of this polypeptide seemed to be higher throughout the entire experiment in WVC lot (Fig. 1c). Additionally, a continuous increase of 26- and 24-kDa band densities was observed from day 0 and from day 5 respectively in FV lot until the end of storage. However, these bands were occurred with lower intensity from the day 5 to the end of storage period in FVC and FVL groups (Fig. 1d). In most cases, the major reported protein changes during storage are weakening of the Z-line, degradation of titin, nebulin, dystrophin and desmin as well as release of  $\alpha$ -actinin from the Z-line, and breakdown of collagen junctions between myotomes (Delbarre-Ladrat et al. 2006). However, the current study divulged small changes in 26–14-kDa area in both SSP and MFP fraction that was relatively concurred with bacterial load rise (day 10) only in WV and FV lots. Furthermore, it was clear that adding cumin or laurel EOs to VP sea bream was speculated to enhance protein degradation. Given the antibacterial property of the considered EOs, this degradation seemed to be caused by endogenous enzymes. Calpain proteases were suggested to be responsible of such proteolysis based on their early post-mortem activation and on their activity enhancement by reducing atmosphere combined with antioxidant treatment. For instance, since desmin (a protein of 55 kDa) was known as calpain substrate, activation of calpain might explain breakdown of this myofibrillar protein which, in turn, may influence muscle-LHC (Huff-Lonergan and Lonergan 2005). In the present work, better information on the presence of such specific proteins and their degradation as a consequence of treatment/storage can be provided by the use of more sensitive techniques based on SDS–PAGE such as immunoblotting study.

#### PCA

With the aim to better understand which factors mostly affect the quality of sea bream batches and to check whether there were correlations between muscle quality parameters, EO treatment and storage time, a PCA model was undertaken on a matrix with 180 objects (sea bream fillets) and 12 variables (pH, TBA, NPS, TMA-N, TVB-N, LL, PBC, MBC, cumin or laurel EO treatment, origin (wild or farmed), and storage time). The fat and moisture contents



**Fig. 1** SDS-PAGE patterns of sarcoplasmic (a–b) and myofibrillar (c–d) proteins extracted respectively from vacuum-packaged wild and farmed *S. aurata* fillet treatments after 0, 5, 10, 15 and 20 days of ice storage. *WV* vacuum-packaged (VP) wild sea bream fillets, *WVC* VP wild sea bream fillets treated with 0.5% cumin EO, *WVL* VP wild sea bream fillets treated with 0.5% laurel EO, *FV* VP farmed sea bream fillets, *FVC* VP farmed sea bream fillets treated with 0.5% cumin EO,

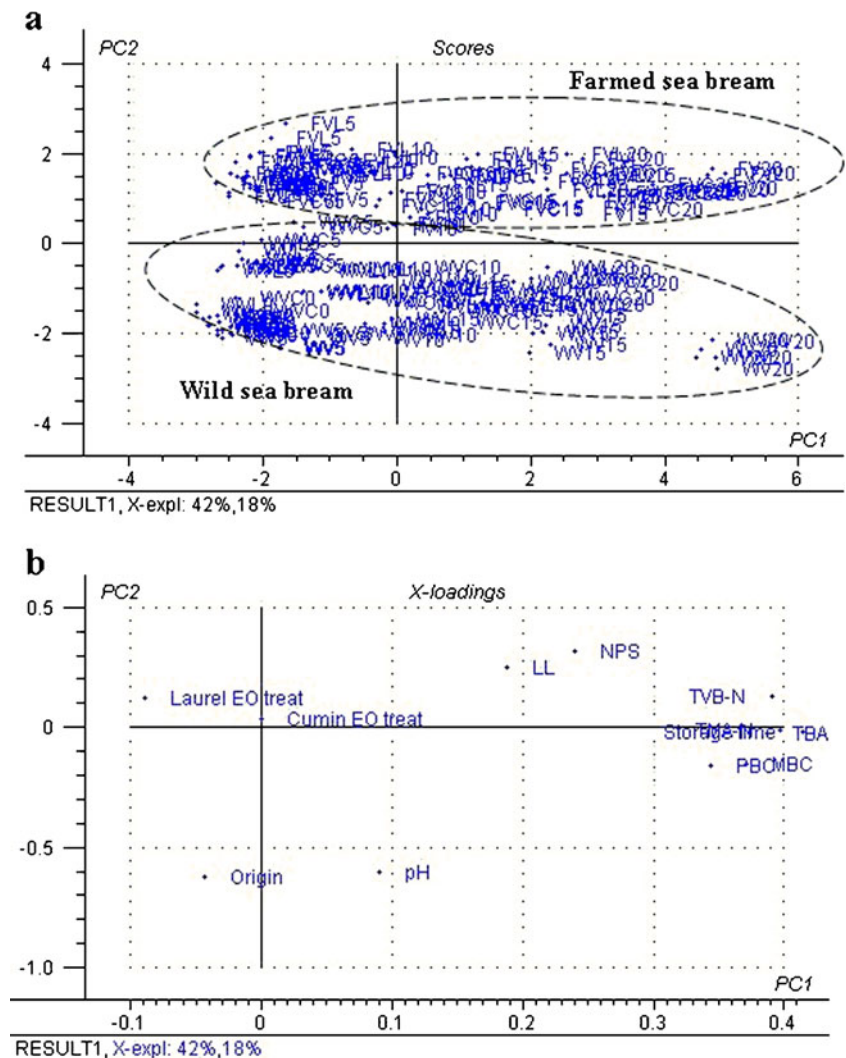
*FVL* VP farmed sea bream fillets treated with 0.5% laurel EO. The numbers above the lanes indicate the days of ice storage. Arrows on the gels indicate the most changed bands throughout storage. *M* protein molecular weight markers, *MHC* myosin heavy chain,  $\alpha$ -*ACN* alpha-actinin, *AC* actin, *TMP* tropomyosin, *MLC* myosin light chain 1 and 2

were not included in the model since they would dominate separation between wild/farmed groups. It was found that two principal components (PCs) explained 60% of the variations in the dataset. The scores and loadings of PC1 and PC2, representing 42% and 18% of the total variation, are given in Fig. 2. The score plot shown in Fig. 2a displayed a clear distinction between wild/farmed sea bream irrespective of fillet treatments. Similar results were recorded for wild/farmed sea bream (Attouchi and Sadok 2010) and for wild/fed cod (Kristoffersen et al. 2006). The discrimination was principally along the principal component two (PC2), where the farmed samples are located in the upper of the score plot and the wild ones in the lower area.

The loading plot (Fig. 2b) shows that the variations in muscle pH are mainly described by PC2 and correlated to whether the fish is wild or farmed. As shown in Fig. 2b, PC1 is spanned out by the parameters laurel EO treatment on the left hand and TVB-N, TMA-N, TBA, PBC, MBC

and storage time on the right-hand sides. Thus, samples with advanced time storage are associated with high level of TVB-N, TMA-N, TBA, PBC and MBC independently of fish origin, while samples treated with laurel EOs are associated with low level of these parameters. Cumin EO treatment appeared to own weaker effect on all considered parameters since it was positioned near the intersection point of the two PC axes. In addition, laurel EO treatment and storage time factors describe a lower influence on LL and NPS attributes than that on other measured attributes. From the loading plot, it is seen that variations in LL and NPS (free amino acids) are described both in PC1 and at lower rank in PC2 and thus both dependent on type of fish and time of storage. Moreover, LL variation was strongly correlated to free amino acid (NPS) production and consecutively to proteolysis activity in all fillet samples. Data from PCA analysis confirmed all acquired results as previously explained.

**Fig. 2** Score (a) and loading (b) plots of the principal component analysis model carried out on all X-variables (pH, TBA, NPS, TMA-N and TVB-N, MBC, PBC, LL, cumin EO treatment, laurel EO treatment, origin (farmed (F) or wild (w)) and storage time). PC1 and PC2 explained 42% and 18% of the variations of the dataset, respectively. Wild and farmed groups are encircled



## Conclusions

Laurel and at a lesser extent cumin EOs exhibited a preservation effect on VP-sea bream fillets during ice storage. Independently of fish origin, lower levels of TVB-N, TMA-N, TBA, PBC and MBC were found in sea bream fillets treated with 0.5% EOs. Thus, the treatment with laurel or cumin EOs extended the shelf life of fish fillets by approximately 5 days in both wild and farmed sea bream compared to nontreated lots. However, EO application seemed to enhance endogenous protease activation, inducing increased muscle proteolysis and in turn a drop in muscle-LHC especially in cultured fish. Such results were confirmed by PCA analysis. The actions of laurel and cumin EOs on structural proteins as well as their synergistic effects were not identified in this study; further investigations on these mechanisms are needed as it would provide insights that may be useful from a technological point of view.

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