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The effect of powdered thyme sprinkling on quality changes of wild and farmed gilthead sea bream fillets stored in ice

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ABSTRACT

The effect of powdered thyme sprinkling on the quality characteristics of fresh and ice-stored wild and farmed gilthead sea bream fillets was assessed. Initially, significant differences in proximate composition and quality attributes were found between wild and farmed fresh sea bream flesh. Throughout ice storage, biochemical alteration appeared more pronounced in farmed fish fillets with significantly higher levels of TVB-N, TMA-N, and TBA; and a lower liquid-holding capacity (LHC). Thyme powder addition (1% w/w) exhibited a preservative effect in both fish lots since significant lower levels of TVB-N, TMA-N, free amino acids (NPS), TBA and LHC were observed in thyme-treated fillets during ice storage. However, thyme inhibitory effect was more marked in wild than farmed fish. As revealed by partial least square regression, LHC in both groups was positively influenced by storage time and trimethylamine accumulation factors, while it was negatively influenced by thyme treatment and fish origin. Hence, LHC was suggested to be related to spoilage bacterial growth. The use of dried thyme extended the shelf life of fish fillets by about 5 days and appeared to be highly valuable to the fish industry as a natural preservative.

1. Introduction

Consumer attitudes towards any food product are a major factor influencing its future development. Thus any new wave of products on the market has to meet the needs of the consumer, such as foods that offer specific health-oriented benefits or ready-to cook food including fish and seafood products. However, due to its high perishability, the quality and shelf life of fish products are restricted and are defined by several factors including both handling practices and origin. For instance, wild fish acceptability is generally higher than cultured fish (Grigorakis, 2007). Thus existing literature noticeably specifies that significant organoleptic differences always exist when comparing wild and cultured flesh of the same species. Among studied parameters, technological quality attributes were found among the most discriminating parameters between wild and reared fish (Mairesse, Thomas, Gardeur, & Brun-Bellut, 2005). Thus, the determination of liquid-holding capacity (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, Olsen, & Ofstad, 2003a). In general, the flesh of farmed fish tends to be softer in texture than wild fish. Therefore it is essential to make an evaluation of high quality fish available to the consumer whether there are differences related to the various post-mortem handling procedure and/or the origin of the fish.

Additionally, and because of its greater awareness and safety concern regarding synthetic chemical additives, food preserved with natural additives has become more popular. For instance, the antimicrobial and antioxidant properties of essential oils, and their active constituents derived from various plant organs have been empirically recognised (Burt, 2004; Lee & Shibamoto, 2002). However, any processing technologies used in the production of such compounds have to prove their technical/scientific efficiencies and the product quality to meet the basic requirement of hygiene and safety standards. It would therefore be economically more convenient to use powdered spices/herbs as ingredients rather their extracts to preserve food including fish fillets (Smid & Gorris, 1999) as they are generally recognised as safe to consume. Beside it is well known that at the core of the traditional Mediterranean cooking, spices and fresh/dried herbs were used for centuries, which current research has confirmed its tremendous health benefits (Burt, 2004; Lee & Shibamoto, 2002). Among the various aromatic plants, thyme (*Thymus vulgaris*) is a characteristic herb of the Tunisian and Mediterranean environment with various beneficial effects, e.g., antiseptic, carminative, antimicrobial, and antioxidative properties (Baranauskiene, Venskutonis, Viskelis, & Dambrauskiene, 2003). In the seafood sector, such preservative compounds including spices/herbs were equally used to extend the shelf life of fish fillets, but at low levels to avoid strong flavours imparting unpleasant sensorial characteristics (Mejlholm & Dalgaard, 2002).

Despite the numerous comparative investigations on post-mortem quality alteration in both wild and cultured sea bream

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through handling, distribution and storage conditions (Alasalvar, Taylor, & Shahidi, 2002, 2005; Grigorakis, 2007; Kuley, Özogul, & Özogul, 2005; Mnari et al., 2007; Özogul, Kuley, & Özogul, 2007), no studies have investigated the changes in liquid-holding capacity in *Sparus aurata* muscle during ice storage.

The aims of the study was to compare the biochemical changes including liquid-holding capacity in wild and farmed gilthead sea bream (*S. aurata*) flesh, and to determinate the effect of dried-powdered thyme (1% w/w) as a natural and low-cost preservative on the quality of sea bream.

2. Materials and methods

2.1. Materials

Thirty cultured gilthead sea bream (*S. aurata*), (average weight and length: 208 ± 22 g and 241 ± 11 mm, respectively) used in this study were bought in January 2005 from the farming unit "Aquaculture Tunisienne", located in Hergla, Tunisia. The commercial feed (Mistral-21, EWOS, Dueñas, Palencia, Spain) used for fish feeding, contained 45% protein, 21% fat, 14.5% carbohydrates, 11% ashes and 1.5% crude fibre. Wild sea bream (30 fishes with average weight and length: 191 ± 18 g and 262 ± 14 mm, respectively) were caught by commercial longline fishing gear during the same day, from the coastal water of the same area (Hergla, Tunisia). The aerial part of wild-thyme (*T. vulgaris*) from Zaghouan Mountain (North of Tunisia), was dried, ground to powder and sterilised by gamma irradiation at 10 kGy (National Center of Nuclear Sciences and Technologies, Sidi Thabet, Tunisia). All chemicals were obtained from Sigma–Aldrich-Fluka Company Ltd. (Poole, Dorset, UK), unless otherwise specified.

2.2. Sample preparation and storage conditions

Wild/farmed gilthead sea bream were killed by immersing in ice cold water (hypothermia) 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), a portion of tissues from each fillet was cut, deep-immersed in liquid nitrogen and stored at −80 °C until proximate/biochemical analysis. The following treatment of fish fillets (n = 30 in each group) were prepared: control lots (W) and (F), and sprinkled with irradiated thyme powder (1% w/w) lots (WT) and (FT). All fillets were packed in polyethylene films (not sealed) separately and than stored in flake 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 15 days. Tissues sampling and storing from each lot was performed as previously reported on days 0, 3, 7, 10 and 15.

2.3. Proximate analysis

The fish samples were analysed for proximate composition: 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 by the Kjeldahl method using potassium sulphate and copper (II) sulphate as the catalysts and 6.25 nitrogen-to-protein conversion factor (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 the Association of Official Analytical Chemists (AOAC, 1995). Lipids were extracted by using chloroform:methanol (2:1 v/v) extraction solution according to the Bligh and Dyer method (Bligh & Dyer, 1959).

2.4. Determination of TVB-N, TMA-N and total free amino acids

For this analysis, 0.5 g of tissue were homogenised (DI-25, IKA, Germany) on ice in 500 µl ultra pure water for 1 min, 0.5 ml of 6% perchloric acid was added and the extract was homogenised for a further 2 min. Homogenates were centrifuged at 14,000g for 20 min and the supernatants were used for total volatile bases (TVB-N) (Ruiz-Capillas & Horner, 1999), trimethylamine (TMA-N) (Sadok, Uglow, & Haswell, 1996) and total free amino acids measured as ninhydrin-positive substances (NPS) (Sadok, Uglow, & Haswell, 1995).

2.5. Determination of thiobarbituric acid value (TBA)

TBA values were determined spectrophotometrically according to the procedure described by Hamre, Næss, Espe, Holm, and Lie (2001). The homogenised sample (0.5 g) was weighed into screw-capped glass tubes and 4.0 ml of chloroform:methanol 2:1 (v/v) with 0.005% butylated hydroxytoluene (BHT) was added. Samples were purged with nitrogen gas; the tubes were closed and incubated with constant shaking for 30 min at room temperature. Thereafter, 2.0 ml of a saturated EDTA solution was added and the tubes were centrifuged for 20 min at 1500g. A 2.0 ml aliquot of the methanol:water layer was transferred to clean screw-capped glass tubes, mixed with 2.0 ml TBA-reagent (1% thiobarbituric acid in 5% trichloroacetic acid) and heated for 30 min at 100 °C. The absorption was measured at 532 nm with a Smart Spec-plus spectrophotometer (Bio-Rad, Hercules, CA) and TBA values quantified by reference to an external standard. The standard was prepared from 50 µl of 1,1- to 3,3-tetraethoxypropan diluted to 50 ml with 0.1N HCl and heated at 100 °C for 10 min. A volume of 2.4 ml of the hydrolysed acetal was then diluted to 100 ml with distilled water, giving a stock-solution equivalent to 0.1 mM malondialdehyde. TBA values were expressed in units of mg malonaldehyde/kg sample.

2.6. pH measurement

Measurement of pH was performed at room temperature using a digital calibrated pH metre (Inolab pH-720, WTW, Weilheim, Germany) on homogenised fillet samples in distilled water (1:2 w/v) (method 981.12, AOAC, 1995).

2.7. Determination of liquid-holding capacity

To evaluate the liquid-holding capacity (LHC) of fillets, a centrifugation test as described by Rørå, Regost, and Lampe (2003) was performed. Chopped frozen muscle samples (2 g) were weighed and placed in a centrifuge tube with weighted ($V1$) filter paper (Whatman, Maidstone, England). Following muscle thawing, the tubes were centrifuged (3K30 Sigma Centrifuge, Osterode am Harz, Germany) at 4000g 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.

2.8. Statistical analysis

All biochemical analysis were carried out in duplicate and averaged for each fillet. For each lot and at each sampling time, the results were presented as mean ± standard deviation of n = 6 fillets. All the data were statistically treated using ANOVA with an SPSS Statistical Software System 11.01 (SPSS Chicago, IL, USA). Tukey's test was used to determine the possible significant differences

among mean values at the 5% level. The results were also analysed by partial least squares (PLS1) regression using multivariate statistical software (The Unscrambler version 9.8, CAMO Software AS, Oslo, Norway). Leverage correction and cross validation of all the data were 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. Martens Uncertainty test was used to examine the influence of considered variables on the LL.

3. Results and discussion

3.1. Proximate analysis

The proximate composition of fresh wild/farmed fish is presented in Table 1. Compared to wild sea bream, the farmed fish flesh had a significantly ($p < 0.05$) higher fat (5.07 ± 0.48% vs. 1.48 ± 0.16% in wild) and lower moisture (73.57 ± 1.25% vs. 78.13 ± 1.46% in wild) contents. Such differences were well established in numerous studies (Flos, Reig, Oca, & Ginovart, 2002; Grigorakis, 2007; Mnari et al., 2007), and have been attributed to the high dietary fat level (21% in this study) in the feed and the reduced activity of farmed fish (Alasalvar et al., 2002). During ice storage, moisture and lipid contents did not show a clear tendency (Table 2), and thyme treatment did not reveal any effect in all sea bream lots. However, in a similar study on farmed sea bream (Kyrana, Lougovois, & Valsamis, 1997); apparent patterns of moisture content variation followed by a reverse change in lipid content were found during ice storage for 24 days.

In this study, protein and ash contents did not differ significantly ($p > 0.05$) between wild/farmed fish lots as it was reported in other studies (Alasalvar et al., 2002; Grigorakis, 2007). In fact, protein and ash contents do not vary as regularly as lipids, since they are mainly determined by the species type, genetic characteristics and size (Morris, 2001). However, in some case muscle crude protein levels can be impacted by dietary protein content and diet composition (Suárez et al., 2009).

Table 1
Proximate composition of Tunisian wild and farmed sea bream fillets. Data are mean ± standard deviation, $n = 6$. Means within the same row with different superscript are significantly different ($p < 0.05$).

	Wild	Farmed
Moisture[A]	78.13 ± 1.46[a]	73.57 ± 1.25[b]
Protein[B]	19.17 ± 0.87[a]	20.11 ± 0.41[a]
Total lipid[C]	1.47 ± 0.16[a]	5.07 ± 0.48[b]
Ash[D]	1.32 ± 0.08[a]	1.39 ± 0.10[a]

[A–D] g/100 g wet fillet.

3.2. Total volatile bases-N variation

The variation of TVB-N contents for all sea bream lots are presented in Table 3. Initially and throughout the refrigerated storage, farmed sea bream had significantly ($p < 0.05$) higher TVB-N contents than wild fish reaching values of 41.57 and 32.92 mg N/100 g, respectively, towards the end of storage. Such difference may be attributed to a higher level of non-protein nitrogenous compounds present in the flesh of farmed gilthead sea bream (Kyrana et al., 1997). It has been shown that initial TVB-N values of a particular fish species is related to the fish non-protein nitrogen content, which in turn depends on fish feeding type, catching season and fish size (Goulas & Kontominas, 2007). Moreover, this change is related to age, locality and culture method (Kyrana et al., 1997). As determined in the different fillet batches, the increase in TVB-N levels followed the order, WT < FT < W < F. At the end of the storage period (day 15), TVB-N values exceeded the upper acceptability limit set by the European commission (CEC, 1995) for TVB-N values of fish (35 mg N/100 g of fish flesh) only in the non-treated farmed lot (F) that reached the value of 41.57 mg N/100 g. As shown in Table 3, powdered thyme treatment produced an obvious preservative effect significantly lowering ($p < 0.05$) the TVB-N levels of both WT and FT fillets as compared with control samples (W and F). Accordingly, the use of dried thyme extended the shelf life of fish fillets by about 5 days in wild and farmed fish. The preservative action of thyme can be attributed mainly to the antibacterial properties of this aromatic plant and more specifically to its phenolic constituents: carvacrol and thymol (Burt, 2004; Mahmoud, Yamazaki, Miyashita, Shin, & Suzuki, 2004). An important characteristic of these phenolic components is their hydrophobicity, which enables their partition within the lipids of the bacterial cell membrane and mitochondria, interfering with the phospholipid bilayer. Such activity disturbs cell structures, which causes an increased permeability and loss of cellular constituents (Burt, 2004; Mahmoud et al., 2004; Mejlholm & Dalgaard, 2002). Another possible route of phenolic compound antimicrobial action is the impairment of a variety of enzyme systems and inactivation or destruction of genetic material of bacteria (Mahmoud et al., 2004). The preservative effect of thyme powder was more pronounced in the WT lot than in the farmed one (FT). The high fat content of cultured *S. aurata* fillets appears to dilute the active components of thyme, mainly thymol and carvacrol and consequently to markedly reduce their antibacterial effectiveness in fish flesh (Burt, 2004). Such a result was comparable with that reported in another study where oregano oil application was found to be more effective in lean fish fillets (cod) than fatty fish such salmon (Mejlholm & Dalgaard, 2002).

Table 2
Changes in the moisture and total lipid of each sea bream fillet groups during storage period. Data are mean ± standard deviation, $n = 6$; Means within the same row with different superscript are significantly different ($p < 0.05$). W, wild sea bream fillets; WT, wild sea bream fillets treated with thyme powder; F, farmed sea bream fillets; FT, farmed sea bream fillets treated with thyme powder.

Days of storage	Analyses	Group W	Group WT	Group F	Group FT
t_0	Moisture[A]	78.13 ± 1.46[a]	78.13 ± 1.46[a]	73.57 ± 1.25[b]	73.57 ± 1.25[b]
	Total lipid[B]	1.47 ± 0.16[a]	1.47 ± 0.16[a]	5.07 ± 0.48[b]	5.07 ± 0.48[b]
t_3	Moisture	78.30 ± 0.71[a]	78.92 ± 0.73[a]	74.88 ± 0.73[b]	73.23 ± 1.31[b]
	Total lipid	1.42 ± 0.17[a]	1.38 ± 0.35[a]	5.52 ± 0.44[b]	5.33 ± 0.44[b]
t_7	Moisture	78.62 ± 1.26[a]	79.53 ± 1.25[a]	74.19 ± 1.01[b]	74.14 ± 0.31[b]
	Total lipid	1.58 ± 0.16[a]	1.43 ± 0.27[a]	5.38 ± 0.40[b]	5.55 ± 0.27[b]
t_{10}	Moisture	79.63 ± 1.02[a]	79.76 ± 1.64[a]	73.80 ± 1.42[b]	73.32 ± 1.38[b]
	Total lipid	1.53 ± 0.19[a]	1.57 ± 0.14[a]	4.32 ± 0.44[b]	5.38 ± 0.54[b]
t_{15}	Moisture	78.92 ± 1.69[a]	78.24 ± 1.50[a]	74.83 ± 1.79[b]	74.13 ± 0.87[b]
	Total lipid	1.48 ± 0.19[a]	1.52 ± 0.21[a]	4.97 ± 0.52[b]	4.93 ± 0.52[b]

[A and B] g/100 g wet fillet.

Table 3
Changes in TVB-N and NPS of each sea bream fillet groups during the storage period. Data are mean ± standard deviation, $n = 6$. Means within the same row with different superscript are significantly different ($p < 0.05$). W, wild sea bream fillets; WT, wild sea bream fillets treated with thyme powder; F, farmed sea bream fillets; FT, farmed sea bream fillets treated with Thyme powder.

Days of storage	Analyses	Group W	Group WT	Group F	Group FT
t_0	TVB-N[A]	8.21 ± 0.91^a	8.21 ± 0.91^a	10.37 ± 0.57^b	10.37 ± 0.57^b
	NPS[B]	0.83 ± 0.08^a	0.83 ± 0.08^a	1.05 ± 0.09^b	1.02 ± 0.09^b
t_3	TVB-N	13.08 ± 0.34^a	13.96 ± 0.70^b	18.73 ± 0.47^c	16.64 ± 0.27^d
	NPS	1.19 ± 0.05^a	1.37 ± 0.16^a	1.34 ± 0.16^a	1.24 ± 0.09^a
t_7	TVB-N	15.09 ± 0.66^a	16.65 ± 0.34^a	20.06 ± 0.78^b	18.76 ± 1.92^b
	NPS	1.26 ± 0.15^a	1.46 ± 0.22^{ab}	1.70 ± 0.24^b	1.22 ± 0.10^a
t_{10}	TVB-N	21.70 ± 0.81^a	18.67 ± 0.94^b	31.10 ± 1.88^c	20.94 ± 0.69^a
	NPS	2.39 ± 0.28^a	1.70 ± 0.17^b	2.50 ± 0.16^a	1.80 ± 0.25^b
t_{15}	TVB-N	32.92 ± 2.92^a	21.70 ± 1.00^b	41.57 ± 3.38^c	27.31 ± 1.57^d
	NPS	3.06 ± 0.27^a	2.07 ± 0.18^b	3.45 ± 0.19^a	2.61 ± 0.24^c

[A] mg TVB-N/100 g.
[B] mmol AA/100 g.

3.3. Free amino acids variation

In this study, the changes in the concentration of FAAs measured as ninhydrin-positive substances (NPS) in all fish lots are shown in Table 3. Initially, farmed sea bream had significantly ($p < 0.05$) higher NPS content than wild sea bream with values of 1.05 and 0.83 mmol AA/100 g, respectively. Over the storage period, the NPS showed significant increases in both (W) and (F) lots mainly towards the end of storage, indicating muscle autolysis. Therefore FAA variation can be used to investigate the effect of factors affecting fish quality as it was suggested in another study (Ruiz-Capillas & Moral, 2001).

Thyme treatment showed a significant inhibitory effect on NPS formation towards the end of storage in both WT and FT groups. Comparable data were reported for aerobically stored minced meat treated with oregano essential oil (Skandamis & Nychas, 2001). Phenolic compounds of oregano oil, mainly thymol and carvacrol (Burt, 2004) as in thyme plant, inhibit bacterial activities (Skandamis & Nychas, 2001).

3.4. Trimethylamine change

The TMA-N values in fish samples are shown in Table 4. The initial TMA-N contents of both wild and farmed sea bream fillets were low with non-significant differences (0.19 and 0.16 mg N/100 g of flesh, respectively) indicating a good fish freshness, and were in the range of TMA-N contents reported in fresh farmed sea bream in other studies (Goulas & Kontominas, 2007). However, in a previous study (Alasalvar et al., 2005), a significant difference in TMA-N content was found between wild/farmed sea bream. A wide range of TMA-N values have been reported to set the acceptability limit: 1 mg N/100 g (Kyrana et al., 1997); 2–3 mg N/100 g (Goulas & Kontominas, 2007); 10–15 mg N/100 g (Connell, 1990). In this study, an increased TMA-N content was recorded throughout the refrigerated storage with the highest level in the 15-days-ice-stored-farmed-fish (2.14 ± 0.08 mg N/100 g), which was, however, within the limit of acceptability. Such low TMA-N levels indicate that the bulk of TVB-N is essentially formed by ammonia as proposed in other study (Kyrana et al., 1997) and that TMA-N is not a useful indicator of freshness in this trial. However, the effect of thyme treatment can be established, as lower values of TMA-N levels were determined in WT and FT during the entire refrigerated storage which may be attributed to antibacterial properties of phenolic compounds of this herb as described above for TVB-N variation.

3.5. Thiobarbituric acid (TBA) variation

TBA changes in sea bream samples are presented in Table 4. A significant TBA increase ($p < 0.05$) was obtained at days 3 and 7 in (F) and (W) groups, respectively, but with significantly lower levels ($p < 0.05$) in wild fish throughout the storage period. These results may be related to several causes, including lower fat content in wild fish as already documented (Simeonidou, Govaris, & Vareltzis, 1998); higher level of $n-3$ polyunsaturated fatty acids in farmed sea bream (Mnari et al., 2007); higher oxidative stress under farming conditions; and the quality of fish feed which may

Table 4
Changes in TBA and TMA-N of each sea bream fillet groups during the storage period. Data are mean ± standard deviation, $n = 6$. Means within the same row with different superscript are significantly different ($p < 0.05$). W, wild sea bream fillets; WT, wild sea bream fillets treated with thyme powder; F, farmed sea bream fillets; FT, farmed sea bream fillets treated with Thyme powder.

Days of storage	Analyses	Group W	Group WT	Group F	Group FT
t_0	TMA-N[A]	0.19 ± 0.08^a	0.19 ± 0.08^a	0.16 ± 0.08^a	0.16 ± 0.08^a
	TBA[B]	0.28 ± 0.06^a	0.28 ± 0.06^a	0.47 ± 0.10^b	0.47 ± 0.10^b
t_3	TMA-N	0.71 ± 0.03^a	0.16 ± 0.03^b	0.52 ± 0.08^c	0.24 ± 0.06^b
	TBA	0.43 ± 0.08^a	0.21 ± 0.02^b	0.75 ± 0.05^c	0.25 ± 0.07^b
t_7	TMA-N	0.92 ± 0.08^a	0.21 ± 0.03^b	0.65 ± 0.05^c	0.26 ± 0.07^b
	TBA	0.80 ± 0.08^a	0.47 ± 0.08^b	0.97 ± 0.04^c	0.38 ± 0.09^b
t_{10}	TMA-N	1.14 ± 0.06^a	0.46 ± 0.20^b	0.83 ± 0.11^c	0.78 ± 0.21^a
	TBA	0.92 ± 0.06^a	0.58 ± 0.06^b	1.73 ± 0.10^c	0.69 ± 0.08^b
t_{15}	TMA-N	1.83 ± 0.17^a	1.02 ± 0.14^b	2.14 ± 0.08^c	1.63 ± 0.17^a
	TBA	1.16 ± 0.08^a	0.76 ± 0.05^b	1.49 ± 0.08^c	0.92 ± 0.07^d

[A] mg TMA-N/100 g.
[B] mg MDA/kg.

contain rancid oil (Murata et al., 1996). TBA levels in (W) and (F) samples increased steadily up to a certain point during storage; followed by either a decrease in values or a lower increase rate. Since the TBA value is a measurement of MDA content, a decrease in MDA may be caused by interaction between MDA and amino acids, proteins, glucose and other fish constituents (Fernandez, Perez-Alvarez, & Fernandez-Lopez, 1997). This observation is in agreement with previous reports (Goulas & Kontominas, 2007). A statistically significant difference ($p < 0.05$) was observed in TBA values of both (WT) and (FT) samples in comparison with (W) and (F) samples, respectively, indicating the strong antioxidant effect of thyme which acts as a radical scavenger. In a previous study, the peroxidation inhibitory activity of ethanol extract of Tunisian thyme powder was determined around 78.07% against α-tocopherol (83.58%), and the radical scavenging activity was IC_{50} = 18.6 μg/ml, which was significantly higher than that of butylated hydroxytoluene (Selmi & Sadok, 2008). According to Connell (1990), TBA values of 1–2 mg MDA/kg of fish flesh are usually regarded as the limit beyond which fish will normally develop an intolerable odour/taste. The TBA values of the present sea bream samples exceeded the value of 1 mg MDA/kg after 10 and 15 days of storage period for (F) and (W) samples, respectively.

3.6. pH variation

Values of pH in all lots are given in Table 5. The initial pH of farmed gilthead sea bream was significantly lower ($p < 0.05$) than wild fish. Similar results were recorded for wild and cultured cod (Kristoffersen et al., 2006; Ofstad et al., 1996; Olsson, Seppola, & Olsen, 2007); for wild and cultured Atlantic halibut (Olsson, Olsen, Carlehog, & Ofstad, 2003b; Olsson et al., 2003a) and for wild and cultured sea bream in a semi-intensive system (Flos et al., 2002). Post-mortem pH can vary from 6.0 to 7.1, depending on several factors including season, species, and feed composition and quantity (Olsson et al., 2007; Simeonidou et al., 1998). In intensive fish farming, the fish is normally fed to satiation to achieve the highest possible growth rate. However, unlimited access to feed leads to increased muscle glycogen and subsequently a low ultimate muscle post-mortem pH due to anaerobic degradation of glycogen (Kristoffersen et al., 2006). The dissimilarity in pH possibly reflects different nutritional states of both fish types, as lower pH values could possibly be due to the higher initial levels of muscle glycogen in well fed farmed fish (Kristoffersen et al., 2006; Kyrana et al., 1997). During storage in ice the pH of control and thyme-treated samples increased at different rates to reach values of 6.80; 6.78; 6.72 and 6.61 for W, WT, F and FT fillet samples, respectively, following 15 days of storage. The increase of pH values during the storage period may be attributed to the production of basic compounds such as ammonia, trimethylamine as well as other biogenic amines by fish spoilage bacteria (Kyrana et al., 1997). Towards the end of storage (days 10 and 15), lower pH values in thyme-treated samples (WT and FT) were recorded in comparison to raw fish samples (W and F) which may be due to thyme inhibitory effects on microbial growth, which delay the formation of basic nitrogen compounds.

3.7. LHC, WHC and fat loss

The changes in fat loss (FL), water loss (WL) and liquid loss (LL) in the different fish fillets lots are given in Table 5. At the beginning of the storage period, FL values were significantly ($p < 0.05$) more pronounced in farmed than wild fish (3.51 and 0.94 g/100 g, respectively). This result may be correlated to the high fat content of farmed gilthead sea bream. However, no correlation was found between muscle fat content and fat loss in Atlantic salmon (Ofstad, Kidman, Myklebust, Olsen, & Hermansson, 1995) or in rainbow trout (Mørkøre, Hansen, Unander, & Einen, 2002). It was equally reported that fat loss increases with storage time as occurred in smoked Atlantic salmon fillets due to collagen denaturation caused by cold-smoking (Rørå et al., 2003). However, such an effect was not noted in this study, as FL levels of all fillet lots were neither affected by thyme treatment nor by storage time in all lots. Hence, the difference of FL levels between wild and farmed fish could be due to a difference in collagen content between both fish groups.

Independently of the storage time, the wild gilthead sea bream fillets had better liquid-holding properties than farmed fish as demonstrated by the elevated ($p < 0.05$) liquid loss of farmed fish at day 0. Similar results were found for water loss in all fresh fish

Table 5
Changes in pH, liquid loss, water loss and fat loss of each sea bream fillet groups during the storage period. Data are mean ± standard deviation, $n = 6$; Means within the same row with different superscript are significantly different ($p < 0.05$). W, wild sea bream fillets; WT, wild sea bream fillets treated with thyme powder; F, farmed sea bream fillets; FT, farmed sea bream fillets treated with thyme powder.

Days of storage	Analyses	Group W	Group WT	Group F	Group FT
t_0	pH	6.61 ± 0.02^{a}	6.61 ± 0.02^{a}	6.38 ± 0.04^{b}	6.38 ± 0.04^{b}
	Liquid loss[A]	16.06 ± 1.10^{a}	16.06 ± 1.10^{a}	20.81 ± 0.79^{b}	20.81 ± 0.79^{b}
	Water loss[B]	15.12 ± 1.23^{a}	15.12 ± 1.23^{a}	17.31 ± 0.82^{b}	17.31 ± 0.82^{b}
	Fat loss[C]	0.94 ± 0.26a	0.94 ± 0.26^{a}	3.51 ± 0.56^{b}	3.51 ± 0.56^{b}
t_3	pH	6.64 ± 0.01^{a}	6.70 ± 0.02^{b}	6.53 ± 0.01^{c}	6.45 ± 0.03^{d}
	Liquid loss	17.63 ± 0.86^{a}	18.32 ± 1.40^{a}	21.91 ± 1.41^{b}	21.42 ± 2.95^{b}
	Water loss	16.74 ± 0.88^{a}	17.45 ± 1.41^{a}	18.19 ± 1.55^{a}	17.55 ± 2.76^{a}
	Fat loss	0.89 ± 0.17^{a}	0.88 ± 0.20^{a}	3.73 ± 0.19^{b}	3.88 ± 0.35^{b}
t_7	pH	6.65 ± 0.01^{a}	6.71 ± 0.01^{b}	6.48 ± 0.01^{c}	6.58 ± 0.03^{d}
	Liquid loss	19.09 ± 1.23^{a}	20.35 ± 0.50^{a}	22.64 ± 0.82^{b}	23.07 ± 0.62^{b}
	Water loss	18.36 ± 1.24^{a}	19.26 ± 0.61^{a}	18.62 ± 0.94^{a}	19.08 ± 0.64^{a}
	Fat loss	0.71 ± 0.19^{a}	1.09 ± 0.34^{a}	4.02 ± 0.42^{b}	3.99 ± 0.27^{b}
t_{10}	pH	6.68 ± 0.01^{a}	6.63 ± 0.01^{b}	6.57 ± 0.01^{c}	6.51 ± 0.02^{d}
	Liquid loss	21.65 ± 0.75^{a}	18.98 ± 2.58^{a}	23.38 ± 0.61^{b}	21.78 ± 2.01^{b}
	Water loss	0.77 ± 0.81^{a}	17.93 ± 2.55^{b}	19.47 ± 0.95^{ab}	18.23 ± 1.66^{ab}
	Fat loss	0.87 ± 0.35^{a}	1.06 ± 0.23^{a}	3.91 ± 0.45^{b}	3.55 ± 0.66^{b}
t_{15}	pH	6.80 ± 0.01^{a}	6.78 ± 0.01^{b}	6.72 ± 0.01^{c}	6.61 ± 0.01^{d}
	Liquid loss	23.03 ± 0.73^{a}	20.19 ± 0.87^{b}	24.45 ± 0.56^{c}	23.16 ± 1.01^{ac}
	Water loss	22.24 ± 0.77^{a}	19.24 ± 0.79^{b}	20.88 ± 0.38^{c}	19.10 ± 1.05^{b}
	Fat loss	0.80 ± 0.18^{a}	0.95 ± 0.36^{a}	3.57 ± 0.46^{b}	4.06 ± 0.63^{b}

[A–C] g/100 g wet fillet.

groups (Table 5). Identical results were reported for wild/semi-intensive system farmed sea bream (Flos et al., 2002), wild/farmed Atlantic halibut (Olsson et al., 2003a, 2003b) and for wild/farmed cod (Ofstad et al., 1996; Olsson et al., 2007). It is equally well known that initial pH values in post-mortem muscle of farmed fish are normally lower than that of its wild counterparts and that low pH negatively influences liquid-holding capacity (Ofstad et al., 1996; Olsson et al., 2003b). In fact, the initial pH affects the volume of the myofibrils in muscle sample and this may fairly explain the disparity in water-holding capacity between farmed and wild fish (Ofstad et al., 1996). Indeed, intensive feeding of fish leads to particularly low ultimate pH, which has been shown to result in low water-holding capacity (WHC) of the muscle (Ofstad et al., 1996). As shown in Table 5, storage time affected liquid-holding capacity of both wild and farmed sea bream fillets. Moreover, LL levels revealed a significant rise ($p < 0.05$) following 7 days of storage in W- and F-lots; and 10 days for WT lot, whereas it remained unchanged in the FT-lot. The farmed sea bream with a different nutritional status, exhibited the greatest variation with storage time. The rate of increase in the LL parameter was significantly ($p < 0.05$) inhibited in thyme-treated fillets; this may be due to the antibacterial effect of this herb as earlier specified. In addition, WL changes were more correlated to LL in all batches (Table 5); hence the sea bream liquid loss increase throughout the ice storage seemed to be more related to water loss than to fat loss.

It was well documented that the liquid-holding capacity of raw muscle decreased with the storage time for several fish species including Atlantic salmon (Rørå et al., 2003) and rainbow trout (Mørkøre et al., 2002). The LHC variations were reported to be more related to muscle pH increase (Kristoffersen et al., 2006). However, results shown in Table 5 for ice-stored and thyme-treated wild and farmed fillets, suggest that other factors than pH are also of importance for LHC variation as equally proposed by Olsson, Ofstad, Lødemel, and Olsen (2003c) who noted that WHC in halibut muscle changes only in the presence of spoilage bacteria. Similarly Olsson et al. (2007) revealed that the WHC of muscle was mostly related to bacterial growth in wild/farmed cod treated with sodium azide (NaN_3) and chilled-stored. Such bacterial growth which is promoted by the farming conditions, affects post-mortem muscle pH interacting with the growth of the H_2S-producting bacteria in cod.

It is worth noting that several parameters have been associated with poor LHC, such as ionic strength, pH, temperature (Ofstad et al., 1995; Olsson et al., 2003b), detachment of sarcolemma, gaps in the extracellular matrix, widening of the intermyofibrillar space, and transversal shrinkage of the muscle fibres (Olsson et al.,

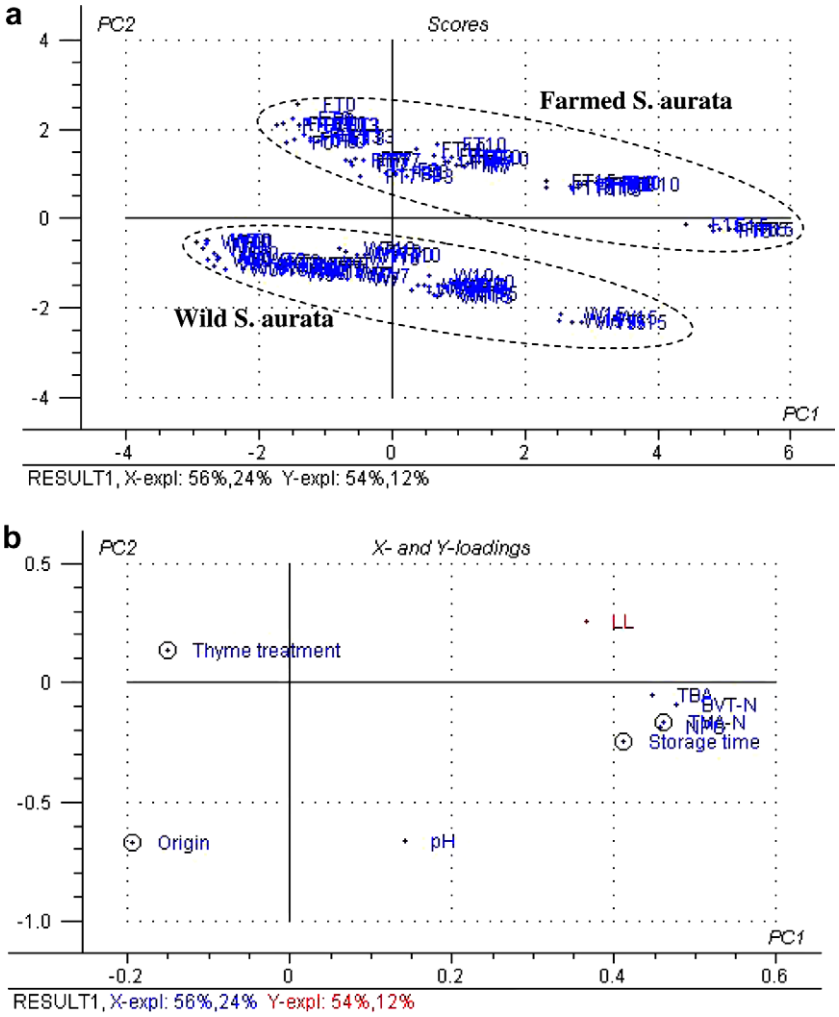

Fig. 1. Score (a) and loading (b) plots from partial least square PLS1 regression carried out on all *X*-variables (pH, TBA, NPS, TMA-N and TVB-N, thyme treatment, origin (farmed (F) or wild (w)) and storage time) and the *Y*-variable (LL). Wild and farmed groups are encircled. Significant variables influencing the LL are represented by point framed by white circle.

2003a). Moreover, given that the majority of the water in the flesh is bound to the myofibrils, and that muscle-WHC is linked to myofibrils-spacing, cathepsins proteases having a significant impact on the protein content could potentially affect the WHC of the muscle (Hagen, Solberg, & Johnston, 2008).

3.8. Partial least square PLS1 regression

In order to find potential influences of pH, TBA, NPS, TMA-N and TVB-N, thyme treatment, origin (farmed or wild) and storage time on the LL, results were explored by the PLS1 model. LL was used as the *Y*-matrix and all the other parameters were used as *X*-matrices. The fat content and moisture were not included in the model since they would dominate separation between wild and farmed groups.

The score plot given in Fig. 1a displayed a clear distinction between wild and farmed gilthead sea bream irrespective of fillet groups. Similar PLS1 results were recorded for fed and wild cod (Kristoffersen et al., 2006). The discrimination was mainly along the principal component two (PC2). The wild samples are located in the lower left of the score plot, the farmed ones in the upper right. Furthermore, results showed that samples with the same storage time tended to be grouped together. The storage groups were oriented in the score plot with increasing days of storage from the left hand side to the right hand side in the principal component one (PC1). Of the total variation in LL, 54% was explained by PC1 and 12% by PC2. Of the total variation in all other variables, 56% was described by PC1 and 24% was described by PC2. As shown in the loading plot (Fig. 1b), PC1 is spanned out by the attributes thyme treatment on the left hand and LL, TBA, TVB-N, TMA-N, NPS and storage time on the right hand side. PC2 mainly describes differences in pH and origin. Sample with high LL are associated with high storage time and subsequently with high level of TBA, TVB-N, TMA-N and NPS, while samples with low LL are associated with low levels of those parameters. By applying the Martens Uncertainty test, the influence of all the variables on the LL was inspected. Results showed that LL was significantly influenced ($p < 0.05$) by four variables, of which thyme treatment and origin were negatively correlated with LL, while TMA and storage time were positively correlated with LL. Such data confirm results previously recorded for the effect of origin, storage time and thyme treatments. On the other hand the positive correlation of LL with TMA may be explained by the decrease of raw quality of fish fillet through storage time due to spoilage bacterial growth as reported by Olsson et al. (2003c, 2007).

4. Conclusions

The parameters that proved to be most sensitive to variations over storage time were FAA, TVB-N concentration and LL.

Significant differences in proximate composition and biochemical parameters exist between fresh wild and farmed gilthead sea bream fillets. Throughout 15 days of ice storage, quality alteration was more pronounced in the farmed than wild fish fillet. Thyme powder treatment (1% w/w) delayed chemical alteration, thereby extending the shelf life of fish fillets of about 5 days. Further studies are needed to identify specific spoilage bacteria in wild and farmed fish that could be responsible for fish quality differences.

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