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Effects of green tea natural extract on quality parameters and lipid oxidation during storage of tench (*Tinca tinca*) fillets

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Summary

The study investigated the effects of two levels of green tea extract (GTE) on common quality parameters and lipid oxidation of tench fillets for up to 7 days of refrigerated (+2°C) and 45 days of frozen storage (−25°C). Sixty-eight tench (93.2 ± 25.7 g) were killed by live chilling (water : ice ratio = 1 : 1; T = 2–4°C) and then filleted. Four fillets were set aside (not treated, NT) while the remaining were divided into four groups which were treated with a sprayed-on solution of: distilled water/ethanol 95% v/v mixture (control group, C); ascorbic acid (AA); and two levels of GTE (0.05% w/v GTE5 and 0.1% w/v GTE10, respectively). Tested were fillet quality parameters (pH, colour, fillets texture and water holding capacity), chemical composition, and lipid oxidation products. Moreover, in NT and refrigerated fillets the total viable counts, Enterobacteriaceae, *Pseudomonas* spp., yeast and moulds microorganisms were assessed. Results showed that GTE at both levels had no negative effects on pH, colour, texture or free water, with results comparable to AA used as a reference synthetic antioxidant. At 7 days of refrigeration, the GTE-treated fillets showed a similar antioxidant capacity of synthetic AA in retarding lipid oxidation. In frozen samples, the best results were obtained by GTE5 treatment while GTE10 seemed to exert a pro-oxidant effect. No significant inhibitory effects of GTE were observed in the microbiological parameters.

Introduction

Tench (*Tinca tinca*) (Linnaeus 1758) is a promising species for aquaculture because of its quality and taste (Gasco et al., 2010). As for other freshwater fish species, the presence of notable concentrations of unsaturated fatty acids makes tench fillets more susceptible to oxidative deterioration (Turchini et al., 2007). To retard lipid oxidation, synthetic antioxidants can be used. Among them, butylated hydroxyanisole (BHA), butylated hudroxytoluene (BHT), tertiary butylhydroquinone (TBHQ) and propyl gallate (PG) have been reported to effectively reduce oxidation (Indrasena and Barrow, 2010). However, the safety of their use is now being questioned because they are considered highly unstable and are suspected as having carcinogenetic and mutagenetic properties. Moreover, consumers are increasingly concerned about the use of synthetic additives in their food. Thus the use thereof is being regulated by governments which regularly review the lists of approved additives and their permitted levels (Shahidi and Wanasundara, 2011; Ortiz et al., 2012).

It has been found that natural extracts from plants and herbs can retard oxidation, improving the shelf life and stability of fish products (Brewer, 2011; Sánchez-Alonso et al., 2011; Farvin et al., 2012; Halldorsdottir et al., 2013; Maqsood et al., 2013).

Green tea (GT) is processed from the leaves of *Camellia sinensis* L., and has been acclaimed for some decades for its remarkable antioxidant features linked to large amounts of tea catechins (TC) (Tang et al., 2001; Perumalla and Hettiarachchy, 2011). These compounds may act as antioxidants by inducing antioxidant enzymes, inhibiting pro-oxidant enzymes or reacting with oxidant agents. In addition to antioxidant properties, TCs extracted from green tea have shown inhibitory effects on positive and negative gram bacteria (Sivarooban et al., 2008). These aspects make GT a good candidate as an antioxidant additive to be used to produce foods with a longer shelf life.

The aim of this study was to investigate the effects of two levels of GT extract on the quality parameters, lipid oxidation and microbiological load of tench fillets during refrigerated (+2°C) and frozen (−25°C) storage.

Materials and methods

Anti-oxidant extractions from green tea

Green Tea Extract (GTE) was obtained according to Jayaprakasha et al. (2001), with slight modifications. Five hundred mg of commercial dried green tea leaves powder (ESI SpA, Albissola Marina, Italy) were mixed with 20 ml of 95% ethanol and shaken with a wrist action shaker for 4 h at 40°C in a water bath. Total phenolic content (TPC) was determined following Chang et al. (2006). A standard curve was evaluated from 0 to 300 μg of gallic acid per ml. The TPC was expressed as mg gallic acid equivalents (GAE) per 100 g of dried sample material. The antioxidant capacity of GTE was measured as free radical scavenging activity according to

Brand-Williams et al. (1995), by an *in vitro* chemical system using the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical. The results were expressed in % of DPPH quenching after 30 min of reaction. Analyses were carried out on three extract samples and the measurements performed in triplicate on each sample (n = 3).

Animals and sampling

A total of 68 tench (*Tinca tinca*) (mean weight 93.2 ± 25.7 g) were killed by live chilling (water : ice ratio = 1 : 1; T = 2–4°C), weighed, carefully eviscerated and immediately filleted by hand. Four fillets were put aside (not treated, NT) while the remaining fillets were weighed and divided into four groups (16 fillets per group) and treated with a sprayed solution of distilled water/ethanol 95% v/v mixture (0.1% w/v, control, C), ascorbic acid (0.1% w/v, AA), green tea extract at lower concentration (0.05% w/v, GTE5), and green tea extract at higher concentration (0.1% w/v, GTE10). All fillets were then vacuum-sealed in oxygen barrier bags. The NT fillets were stored at +2°C and analysed after 1 day of storage while the treated fillets were cold stored at +2°C or frozen at −25°C and picked for analyses at 3 and 7 days of cold storage and at 30 and 45 days of frozen storage, respectively. In all groups, all right fillets were used for the determination of pH, colour, chemical composition, lipid oxidation products and microbiological load, while left fillets were used for texture and water holding capacity (as free water, FW) determinations. For each sampling time and for each fillet (n = 4), analyses were performed in triplicate, with the exception of chemical analyses that were performed in duplicate.

Quality parameters analyses

In all fillets, at three different points of the epaxial muscle the parameters assessed were:

1. pH, using a Crison MicropH 2001 (Crison Instruments, Barcelona, Spain) equipped with a combined electrode and an automatic temperature compensator;
2. colour, using a Minolta CR-331C Minolta Colorimeter (∅ 25 mm measuring area, 45° circumferential illumination/0° viewing angle geometry) with the D65 illuminant and 2° standard observer. Results are expressed in terms of lightness (L*), redness (a*) and yellowness (b*) in the CIELAB colour space model (Commission Internationale de l'Éclairage (CIE), 1976);
3. texture, using a Zwick Roell® texturometer equipped with a 200 N load cell. A one cycle compression test was done by using a 10 mm diameter cylindrical probe (at a constant speed of 30 mm min^{-1} until 50% total deformation);
4. water-holding capacity (determined as free water, FW), by applying the compression test (Grau and Hamm, 1953).

Chemical composition and lipid oxidation

After the previously listed analyses, fillets were freeze-dried for the other analysis assessments.

Chemical composition analyses were expressed on a fresh weight basis (A.O.A.C., 2000). The extent of lipid oxidation was determined at 3 and 7 days (refrigerated fillets) and at 30 and 45 days (frozen fillets) only on treated fillets (C, AA, GTE5, and GTE10) by the thiobarbituric acid reactive substance (TBARS) assay modified from Witte et al. (1970). Four fillets (n = 4) were analysed for each treatment and sampling time and each fillet was analysed in triplicate. Results were expressed as mg malondialdehydes (MDA) kg^{-1} of muscle, using a standard curve of 1,1,3,3-tetramethoxypropane (Sigma–Aldrich, Steinheim, Germany).

Microbiological analysis

At the beginning on NT fillets and on days 3 and 7 of the refrigerated storage, four fish per group were microbiological analysed according to the standard procedure (ISO, 2003a). From the resulting dilution, appropriate decimal dilutions were prepared, using the same diluents and plated in duplicate to enumerate the following microorganisms:

1. total viable counts (TVC), by the pour-plate method using Plate Count Agar. Plates were incubated at 30°C for 48 h (ISO, 2003b);
2. Enterobacteriaceae, by the inclusion method using VRBG agar. Plates were incubated at 37°C for 24 h (ISO, 2004);
3. *Pseudomonas* spp., using the surface-plate method on *Pseudomonas* agar base. Plates were incubated at 30°C for 48 h (ISO, 2010);
4. Yeast and moulds, using the surface-plate method on Sabouraud Dextrose Agar. Plates were incubated at 25°C for 72–110 h (ISO, 2008).

Statistical analysis

Physical traits, microbial counts, and TBARS values were analysed independently in each refrigerated and frozen storage time by one-way ANOVA (treatment as categorical variable) (R, 2005). The NT group was included each time in the analysis with the exception of the TBARS models. When ANOVA was significant, a pairwise comparison was performed with Duncan's test. Moreover, Student's *t*-tests for independent samples were performed in order to compare the means of the same treatment between the refrigerated and frozen storage times. Values were reported as means of group ± standard error of the mean. For all tests, significance was accepted for $P < 0.05$.

Results

Total phenolics and antioxidant activity

The total phenolic content of GTE was 96.6 mg gallic acid equivalents (GAE) per 100 g of product and its antioxidant activity was 89.27 ± 0.10% by the DPPH method.

Quality parameters

The pH, colour, texture and free water results of NT, and treated fillets during refrigerated and frozen storage are reported in Table 1, respectively.

Table 1
Effect of antioxidant treatments on physical traits of tench fillets during (a) cold storage at +2°C and (b) frozen storage at −25°C (mean ± standard error of the mean, n = 4)

(a)

Days of storage	1	3				7				Student's *t* –test
Treatment[1]	NT	C	AA	GTE5	GTE10	C	AA	GTE5	GTE10	3 vs 7
pH	6.23 ± 0.05B	6.16 ± 0.03B	6.18 ± 0.03B	6.34 ± 0.05A	6.21 ± 0.03B	6.20 ± 0.03	6.17 ± 0.02	6.24 ± 0.04	6.19 ± 0.03	–
L* (lightness)	47.39 ± 0.99CY	51.80 ± 1.16AB	52.94 ± 0.94A	49.04 ± 0.75BC	50.27 ± 1.46ABC	54.98 ± 1.57X	54.32 ± 0.74X	52.23 ± 1.45X	51.95 ± 1.34X	–
a* (redness)	−1.08 ± 0.28Y	−0.77 ± 0.22	−0.28 ± 0.39	−0.53 ± 0.50	−0.79 ± 0.41	1.45 ± 0.67X	−0.91 ± 0.20Y	−0.17 ± 0.44Y	0.16 ± 0.44XY	*C
b* (yellowness)	−1.22 ± 0.34BY	−0.39 ± 0.28AB	0.84 ± 0.56A	0.03 ± 0.56AB	0.09 ± 0.34AB	2.12 ± 0.84X	1.12 ± 0.43X	1.73 ± 0.62X	1.88 ± 0.60X	**C, GTE10
Texture (N)	8.82 ± 0.53	7.89 ± 0.24	8.61 ± 0.30	8.32 ± 1.0	7.17 ± 0.69	7.53 ± 0.89	7.66 ± 0.77	8.90 ± 0.75	6.61 ± 0.40	–
FW (cm^2)	13.40 ± 1.13AY	9.62 ± 1.37B	15.93 ± 0.59A	15.30 ± 0.46A	15.25 ± 0.07A	17.18 ± 0.90X	18.44 ± 0.79X	17.89 ± 0.65X	17.40 ± 0.68X	***C, GTE5

(b)

Days of storage	1	30				45				Student's *t* –test
Treatment[1]	NT	C	AA	GTE5	GTE10	C	AA	GTE5	GTE10	30 vs 45
pH	6.23 ± 0.05X	6.18 ± 0.04	6.30 ± 0.05	6.24 ± 0.03	6.24 ± 0.03	5.97 ± 0.03XY	6.10 ± 0.07XY	6.03 ± 0.05XY	5.95 ± 0.04Y	–
L* (lightness)	47.39 ± 0.99C	53.53 ± 0.74A	50.65 ± 0.81B	53.49 ± 1.25A	55.53 ± 0.75A	49.30 ± 1.15	47.53 ± 1.17	46.66 ± 0.63	46.36 ± 0.54	–
a* (redness)	−1.08 ± 0.28BC	−0.17 ± 0.40AB	0.37 ± 0.58A	−0.28 ± 0.29AB	−1.43 ± 0.16C	−0.47 ± 0.17	−0.48 ± 0.29	−0.25 ± 0.32	−0.77 ± 0.29	*C
b* (yellowness)	−1.22 ± 0.34BY	2.30 ± 0.31A	2.21 ± 0.48A	2.67 ± 0.38A	2.93 ± 0.48A	1.09 ± 0.46X	−0.02 ± 0.32X	0.97 ± 0.23X	0.34 ± 0.38X	**C, GTE10
Texture (N)	8.82 ± 0.53A	7.86 ± 0.74AB	9.72 ± 0.73A	6.20 ± 0.41B	8.46 ± 0.68A	9.35 ± 1.64	6.45 ± 0.57	8.41 ± 1.01	6.83 ± 0.65	†AA
FW (cm^2)	13.40 ± 1.13AX	6.92 ± 0.31 BC	6.52 ± 0.88C	8.44 ± 0.69BC	9.20 ± 0.61B	6.72 ± 1.11Y	8.68 ± 1.60Y	8.34 ± 0.30Y	7.68 ± 0.39Y	–

[1]NT, Non-Treated group; C, control group; AA, Ascorbic Acid group; GTE5, Green Tea Extract 0.05%; GTE10, Green Tea Extract 0.1%.
*C = significant Student's *t*-test for Control group.
**C, GTE10 = significant Student's *t*-test for Control and Green Tea Extract 0.1 % group.
***C, GTE5 = significant Student's *t*-test for Control and Green Tea Extract 0.05% groups.
†AA = significant Student's *t*-test for Ascorbic Acid group.
Different letters (A, B, C) in same row mean significant differences among non-treated group and each treated group at (a) 3 days and (b) 30 days of storage.
Different letters (X, Y) in the same row mean significant differences among non-treated group and each treated group at (a) 7 days and (b) 45 days of storage.

Considering the pH of refrigerated fillets, significant differences appeared among NT and treated groups after 3 days of storage, with the highest value (6.34) recorded in the GTE5 group; at 7 days of storage no differences appeared. On the other hand, in pH of frozen fillets significant differences appeared among NT group and treated groups after 45 days of storage, with the lowest value (5.95) recorded in the GTE10 group.

In both types of conservation the number of days of storage had no different effects, as shown by the Student's *t*-test when comparing treated groups to each other.

In refrigerated fillets all colour parameters were significantly influenced by the treatment, with the exception of redness at 3 days of storage. Values of all parameters, when compared with the NT group, increased with the days of storage.

In frozen fillets, lightness and redness showed statistical differences only at 30 days of storage, while the yellowness was statistically different at both times of storage. Moreover, in both types of conservation the days of storage did not influence lightness, while statistical differences appeared for redness in the control group. The Student's *t*-test showed differences for yellowness in the control and GTE10 groups.

As far as texture is considered, in refrigerated fillets no differences were found during the storage period, whereas differences appeared among NT and treated groups at 30 days of frozen storage, with the lowest value shown in the GTE5 group (6.20).

Student's *t*-test was significant only in frozen fillets for the AA group (9.72 vs 6.45 for 30 and 45 days, respectively).

Water holding capacity highlighted differences both at 3 and 7 days. The FW values increased with the time of storage, except in the C group at 3 days that showed the lowest value (9.62). An opposite trend was observed in frozen fillets, with a general decrease in NT and treated groups. Among treatments, at 30 days GTE10 and AA groups showed the highest (9.20) and lowest (6.92) values, respectively, while at 45 days no differences appeared. Student's *t*-test was significant only in refrigerated fillets for C and GTE5 groups, with higher values recorded at 7 days.

Chemical composition and lipid oxidation

No differences appeared in the chemical composition of fillets analysed at different storage times. Analysed parameters showed the average values of dry matter 27.2 ± 2.05, crude protein 19.7 ± 0.24, ether extract 3.24 ± 0.57, and ash 1.25 ± 0.14.

Results of the TBARS analysis of refrigerated and frozen fillets are reported in Fig. 1a and b, respectively. For the refrigerated trial, at 3 days of storage, fillets of AA (0.73) and GTE10 (0.75) groups highlighted significantly lower values than C (0.93) and GTE5 (1.0). After 7 days, the C group reported the highest values (0.79), whereas the other groups showed similar values ranging from 0.51 and 0.57 mg MDA/kg muscle.

In frozen samples at 30 days of storage the GTE5 group showed the significantly lowest value (0.06). Moreover, after 45 days, GTE5 remained the group with the lowest values (0.34); however, these differences were significant only with regard to the C (0.63) and GTE10 (0.86) groups.

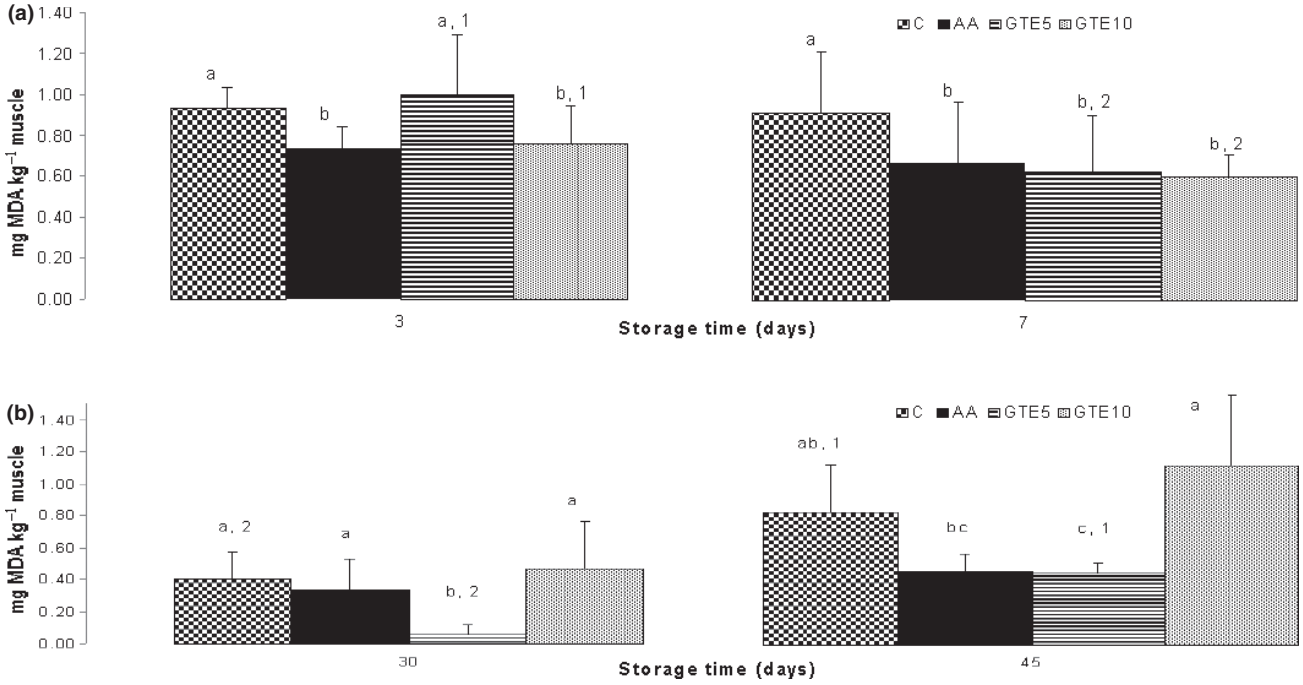

Fig. 1. (a) Formation of malondialdehydes (MDA) in treated tench fillets during refrigeration storage at +2°C (mean ± standard deviation, n = 4). (b) Formation of malondialdehydes (MDA) in treated tench fillets during frozen storage at −25°C (mean ± standard deviation, n = 4). Values without a common alphabetical letter among treatments within each storage time indicate a significant difference ($P < 0.05$). Values without a common number between storage times are different ($P < 0.05$). (Student's *t*-test, $P < 0.05$).

Microbiological analysis

The microbiological results are reported in Table 2. The TVC number at 3 days was not different from the value of the NT group, whereas the values increased significantly after 7 days of storage. As far as Enterobacteriaceae are concerned, differences appeared in both days of refrigeration when comparing treated and NT groups. Among treatments, only at 3 days did a difference appear between AA and GTE5 groups. *Pseudomonas* spp. was not found in NT fillets; values ranging from 2.04 and 3.79 log CFU g^{-1} were found at 3 days with no differences among treatments, while at 7 days the values increased, reaching significant differences between C (4.43) and GTE5 (5.08). Yeast and moulds showed an increasing trend during the storage time, but with significant differences only after 7 days. Student's *t*-test showed differences for all microbial analyses in all groups, except for the GTE5 group in yeast and moulds.

Discussion

The TPC of GTE (96.6 mg GAE 100 g^{-1}) found in the product used in this trial was higher than that reported by Chan et al. (2007), who found values of 21 mg GAE 100 g^{-1} in tea extract. The antioxidant activity of green tea is attributed to the presence of phenolic hydroxyl groups in TC that can interact with the free radicals to inhibit lipid oxidation (Mitsumoto et al., 2005) and to its metal-chelating features (Kumamoto et al., 2001). The antioxidant activity of GTE, obtained by the DPPH quenching method, was 89.27%. Comparing this value with another natural plant extract, GTE appeared to have a higher antioxidant activity than grape seed (Kim et al., 2006).

The NT fillets showed a pH of 6.2, while 24 h after death in different genetic strains of tench, Wedekind et al. (2003) reported data ranging from 6.40 to 6.52. In treated fillets pH ranged between 6.16 and 6.34 for refrigerated fish. Therefore, experimental treatments did not affect the flesh pH during storage. Some authors reported a pH limit of acceptability of 6.8–7.0 (Erkan et al., 2011). In the present study pH values were always under this limit. During storage at −25°C, the pH values were affected by treatments and highlighted differences after 45 days, showing a general decrease with the lowest value in fillets treated with GTE10.

Colour parameters were evaluated to detect whether antioxidant treatments caused any changes in the treated fillets. In refrigerated fillets, all colour parameter values increased in all treatments with the length of the storage period, except for the redness value of the AA-treated group that decreased from −0.28 to −0.91 at 7 days of storage. Negative values of redness and yellowness were found in bluefish flesh treated with thyme and laurel essential oil (Erkan et al., 2011) or in fillets of gilthead sea bream fed with plant protein mixture (De Francesco et al., 2007). Many plant extracts have a dark colour, which may affect the appearance of the final product. The degree of colour change depends on the extract colour and level, as reported for sturgeon fillets tumbled in green tea extract (Haghparast et al., 2011); they explained this result as the possible penetration of tea chlorophyll pigments

Table 2
Antimicrobial effects of antioxidant treatments on the microbiological load (log CFU/g) of tench fillets during cold storage at +2°C (mean ± standard error of the mean, n = 4)

Days of storage	1	3				7				Student's *t* –test*
Treatment[1]	NT	C	AA	GTE5	GTE10	C	AA	GTE5	GTE10	3 vs 7
Total Viable Counts	3.45 ± 0.15Y	3.66 ± 0.14	3.24 ± 0.20	4.00 ± 0.16	3.62 ± 0.18	4.87 ± 0.32X	5.22 ± 0.18X	5.21 ± 0.09X	4.79 ± 0.18X	C,AA,GTE5, GTE10
Enterobacteriaceae	0.50 ± 0.29CY	2.68 ± 0.03AB	1.58 ± 0.54B	2.84 ± 0.15A	2.31 ± 0.44AB	4.14 ± 0.23X	4.40 ± 0.15X	4.44 ± 0.14X	4.37 ± 0.06X	C,AA,GTE5, GTE10
Pseudomonas spp.	0.0 ± 0.0BZ	2.04 ± 0.73A	2.88 ± 0.27A	3.79 ± 0.21A	2.45 ± 0.93A	4.43 ± 0.33Y	4.78 ± 0.27XY	5.08 ± 0.08X	4.99 ± 0.10XY	C,AA,GTE5, GTE10
Yeast and moulds	1.00 ± 0.58Y	2.29 ± 0.18	1.80 ± 0.63	2.25 ± 0.78	2.65 ± 0.38	3.56 ± 0.24X	3.60 ± 0.02X	3.81 ± 0.08X	3.62 ± 0.01X	C,AA,GTE10

[1]NT, Non-Treated group; C, control group; AA, Ascorbic Acid group; GTE5, Green Tea Extract 0.05%; GTE10, Green Tea Extract 0.1%.
*C,AA,GTE5,GTE10 = significant Student's *t*-test for Control, Ascorbic Acid, Green Tea Extract 0.05% and Green Tea Extract 0.1% groups, respectively.
Different letters (A, B, C) in the same row mean significant differences among non-treated group and each treated group at 3 days of storage.
Different letters (X, Y, Z) in the same row mean significant differences among non-treated group and each treated group at 7 days of storage.

that caused an undesirable change of colour in the flesh. In our samples, colour values followed a similar trend in changes of colour parameter values, as was also found in the minced muscle of chub mackerel added with plant extracts obtained from pomegranate and grape seed extracts during frozen storage (Özalp Özen et al., 2011).

The muscle texture of fish depends on intrinsic biological factors (content of fat and collagen) and on autolytic and microbiological processes triggered after the death of the fish, and which are responsible for the degradation of myofibrillar protein and eventual muscle softening. In these trial findings, the values registered for texture of refrigerated fillets were similar in all treatments, at both times of storage, and with minimal changes also found in frozen fillets. The expected reduction of hardness due to freezing was only sporadically found in fillets differently treated and not systematically confirmed at both times of storage.

Results indicated that texture changes during refrigerated or frozen storage were minimal, both in fillets treated with antioxidants and in the C fillets, suggesting an inconsistency with other research findings that have shown fish to become less firm with longer chilling/freezing periods (Rawdkuen et al., 2010).

The water-holding properties are greatly influenced by structural changes in the muscle proteins during storage. An increase of expressible moisture is due to the reduction of the muscle water-holding capacity as a consequence of protein denaturation (Suvanich et al., 2000). This behaviour could explain the increase of FW values registered in refrigerated fillets stored for 7 days, in comparison to the NT fillets that were analysed after 1 day of storage. C and GTE5 fillets have undergone a significant increase in the quantity of water released from the muscle with the progress of the duration of storage, as evidence of a likely greater muscle denaturation during the shelf life.

However, the overall behaviour of refrigerated fillets treated differently was quite similar, analogously to the frozen fillet after 45 days of storage. The differences in FW among treatments in frozen fillets after 1 month of storage (the lowest values for AA and highest for GTE10) are not clear since they were not confirmed after the longer storage period. No significant differences were detected by Rostamzad et al. (2010) in expressible moisture content among fillets of Persian sturgeon treated with ascorbic and citric acid during 6 months of frozen storage; similar results were also reported by Pourashouri et al. (2009) on wells catfish. Conversely to the findings of Rostamzad et al. (2010), in our trial a relevant decrease of FW was observed in frozen fillets in comparison to the NT fillets, with no changes due to storage duration. Probably the small size of the tench fillets had fostered a more natural release of water directly lost by the fillets as a result of freezing.

Values found for the chemical composition were similar to previous data (Gasco et al., 2010) obtained from fish from the same pond farm. Crude protein content was slightly higher compared with results obtained by other authors (Turchini et al., 2007). As regards to ether extract content, it is well known that several factors, diet above all, affect fat content (Gasco et al., 2010). Present data from fillets from tench reared in a semi-intensive conditions with natural food supplemented with a formulated diet were in accord with those of Özogul et al. (2007).

After 3 days of refrigerated storage, the highest oxidative rancidity, as measured by TBARS (Fig. 1a), was found in C and GTE5 samples, while after 7 days the values reported by AA, GTE5 and GTE10 were statistically lower than in the C group. In frozen samples, only GTE5 was efficient in lessening the lipid oxidation at both 30 and 45 days, while GTE10 highlighted values comparable to the C group.

These results only partially agreed with the observations of Alghazeer et al. (2008), who found the highest TBARS values in mackerel without antioxidant after 8 weeks of storage. On the other hand, Tang et al. (2001) reported that TC should be added at concentrations greater than 300 mg kg^{-1} in order to reduce lipid oxidation in mackerel patties.

Yerlikaya and Gokoglu (2010) reported that, even when oxidation increased progressively through the frozen storage in bonito fillets treated with green tea and grape seeds extracts, the values of samples treated with natural antioxidant extracts remained at low levels. Both plant extracts displayed successful effects in delaying lipid oxidation compared to the control groups. In our trial, only GTE5 showed this effect. It could be hypothesised that the GTE10 concentration was too high, leading to a pro-oxidant effect as reported by Ojagh et al. (2011). Chinese green tea extracts showed strong inhibitory effects on major foodborne pathogens in vitro and in vivo studies, as recently reviewed by Perumalla and Hettiarachchy (2011). Nevertheless, in our trial this effect was not confirmed on the tested strains.

Due to their unsaturated fatty acid contents, fish are well known to be susceptible to oxidation, a process that leads to an off-flavour and loss in nutritive value. The results obtained in our trial showed that GTE had no negative effects on physical traits and resulted as being comparable to AA used as reference synthetic antioxidant.

At 7 days of refrigeration, the GTE-treated fillets showed a similar antioxidant capacity of synthetic ascorbic acid in delaying lipid oxidation when compared to the control group. In frozen samples, the best results were obtained with the GTE5 treatment while higher levels of green tea extract seemed to exert a pro-oxidant effect.

Concerning microbiological aspects, our experiment results do not highlight any significant inhibitory effects of natural extracts.

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