



The effect of natural antioxidant (*Thymus vulgaris* Linnaeus) on flesh quality of tuna (*Thunnus thynnus* (Linnaeus)) during chilled storage

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Abstract. Tuna *Thunnus thynnus* flesh was treated with dried thyme *Thymus vulgaris*, vacuum-packed and then stored at 0 °C for 18 days. Proximate composition, thiobarbituric acid reactive substance (TBARS), total volatiles bases, trimethylamine, pH and fatty acids composition were determined throughout the period of storage. No significant differences ($p > 0.05$) between moisture, ash, protein and lipid were found between lots. The antioxidant effect of thyme was shown by the lower TBARS levels and the unaffected fatty acids composition found in thyme-treated lot. Significant differences ($p < 0.05$) were observed in fatty acid profiles of control lot before and after storage, whereas no significant differences were recorded for treated lot in any class of fatty acids after 15 days of storage. In control samples, saturated and monounsaturated fatty acids percents increased from 30.66 to 34.16 and from 20.33 to 21.32 respectively, whereas the polyenes percent decreased from 41.32 to 38.64%.

Key words: Tuna, Thyme, Antioxidant, Chilling, Oxidation, TBARS, Fatty acids.

Resumo. O efeito de antioxidantes naturais (*Thymus vulgaris* Linnaeus) na qualidade da carne de atum (*Thunnus thynnus* Linnaeus) durante o armazenamento refrigerado. A carne do atum *Thunnus thynnus* foi tratada com tomilho seco *Thymus vulgaris*, embalada a vácuo e, em seguida, armazenado a 0°C durante 18 dias. Composição centesimal, substâncias reativas ao ácido tiobarbitúrico (TBA), bases voláteis totais, trimetilamina, pH e a composição de ácidos graxos foram determinados durante todo o período de armazenamento. Nenhuma diferença significativa ($p > 0,05$) entre a umidade, cinza, proteína e lipídio foram encontrados entre os lotes. O efeito antioxidante do tomilho foi demonstrado pelo menor nível de TBA e os níveis inalterados da composição de ácidos graxos composição foram encontrados no lote tratado com tomilho. Diferenças significativas ($p < 0,05$) foram observadas nos perfis de ácidos graxos do lote controle antes e após o armazenamento, enquanto que nenhuma diferença significativa foi registrada para o lote tratado em qualquer classe de ácidos graxos após 15 dias de armazenamento. Nas amostras controle, os percentuais de ácidos graxos saturados e monoinsaturados aumentaram de 30,66 para 34,16 e de 20,33 para 21,32, respectivamente, enquanto que o percentual de polienos diminuiu de 41,32 para 38,64%.

Palavras-chave: Atum, tomilho, antioxidante, resfriamento, oxidação, TBA, ácidos graxos.

Introduction

Tuna is an important fish species with high economic value as a result of its growing demand in international markets. It is generally consumed fresh, canned and frozen. Fish lipids are known to be rich in polyunsaturated fatty acids (PUFAs), especially the n-3 PUFAs family including eicosapentaenoic acid (EPA or 20:5 n-3), docosapentaenoic acid

(DPA or 22:5 n-3) and docosahexaenoic acid (DHA or 22:6 n-3) which may readily be oxidised to form lipid hydroperoxides (Osman *et al.* 2001, Boran *et al.* 2006). Oxidation of lipids that occurs in raw material during storage, processing, heat treatment, and in the final products during subsequent storage, is one of the basic processes causing rancidity in

food products (Donelli & Robinson 1995). Such oxidative deterioration may affect the organoleptic characteristics, including taste and aroma making the final product unacceptable for consumption. Therefore, several investigations have been undertaken with the aim to enhance the shelf-life extension, the stability of lipid-containing products and food quality. Application of antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) in the food industry is one of the most technically and commonly used procedures to reduce the oxidation process (Frankel 1993, Karpinska *et al.* 2001). However, such substances are volatile and easily decompose at high processing temperatures. The possible toxicity of synthetic chemicals used as antioxidants has been the subject of several studies (Powell *et al.* 1986, Thompson & Trush 1986) and recently, the food industry and nutritional institutes (Food Science Australia, Riverside Life Science Centre, Australia; Bioanalytical Laboratory, Pharmaceutical Product Development Inc., Middleton, Wisconsin, USA ; Institute of Organic Chemistry, Bulgarian Academy of Sciences, Sofia, Bulgaria...) focused investigations on the use of natural antioxidants (spices, herbs, and vegetable extracts) due to their antioxidant properties and phenolic contents (Akhtar *et al.* 1998). For instance, the antioxidative effect of thyme is based essentially on polyphenolic compounds as flavonoids (Luteolin), while thymol and carvacrol have low activity (Hollman *et al.* 1996, Lacroix *et al.* 1997, Justesen & Knuthsen 2001).

Despite the numerous cited studies on the antibacterial and antioxidant activity of herbs and vegetable extracts (Chaieb *et al.* 2007) and subsequent effect on the shelf life of foods, few data exist on the effect of *Thymus vulgaris* on the shelf-life of refrigerated fish and fish products. The objective of the present work was to study the combined effect of vacuum-packaging and antioxidant activity of *Thymus vulgaris* on the shelf-life and quality indicators of tuna fillets during 18 days of chilled storage.

Material and Methods

Fish sampling and processing

Tuna *Thunnus thynnus* was caught in April 2006 in the Northern area of the Tunisian Sea. The fishes were transported on ice to the laboratory of Department Marine Biodiversity and Biochemistry, INSTM, (La Goulette, Tunisia) where they were eviscerated, headed and filleted. Thyme *Thymus vulgaris* from Tunisia was dried, powdered and sterilised by a dose of 25 kGy

of gamma irradiation in the National Centre of Nuclear Sciences and Technology (Sidi Thabet, Tunisia). Tuna fillets (150 ±10g, each) were separated into two lots: one was left as a control lot and the second was sprinkled (0.1%) with powdered thyme. All lots were then vacuum-packed in polyethylene bags and stored at 0 °C for 18 days.

Tissue sampling was performed on days 0, 3, 6, 9, 12, 15, 18. At each time, samples were rapidly stored at -80 °C until analysis. Frozen period was no longer than 3 weeks. Prior to analysis, tuna flesh was thawed and homogenized on ice for 3 min using an Ultra-turrax blender. Sample homogenates were immediately subjected to analysis.

Biochemical analysis

Moisture

Moisture of the tuna samples was determined according to the AOAC (1990) method by drying in an oven at 105 °C (n = 6). Results were expressed as percentage of wet weight.

Ash

Ash content was determined by heating sample for 6 h in an oven at 525 °C (n = 6) according to the AOAC (1995) method. Results were expressed as percentage of wet weight.

Protein

Total protein content in the homogenized samples was determined using the method described by Lowry *et al.* (1951). This colorimetric method uses the fact that the Folin-Ciocalteu reagent in presence of protein, was reduced in a blue complex which the intensity was directly proportional to the protein content, and showing a maximum of absorbance to 750 nm. Results were expressed as g per 100 g of fresh sample.

Carbohydrate

The carbohydrate content was determined according to the method of Dubois *et al.* (1956). Simple sugars, oligosaccharides, polysaccharides, and their derivatives give an orange-yellow color when treated with phenol and concentrated sulphuric acids. Results were expressed as mg per 100 g of fresh sample.

Lipids extraction

Total lipids were extracted according to the method of Folch *et al.* (1957) by chloroform/methanol (2/1). The aliquot of the chloroform layer extract was evaporated to dryness and lipids were quantified gravimetrically.

Fatty acids analysis

Fatty acids Methyl Esters (FAMES) were obtained by the method described by Metcalfe *et al.* (1966). A fraction of the lipid extract was saponified with 0.5 mol/L NaOH in methanol followed by a methylation in 12% boron trifluoride in methanol (BF₃/MeOH). The methylated sample was then extracted with n-hexane. All of these reactions were performed in quadruplicate for each sample. The resulting methyl esters were analysed by gas chromatography (GC) using an Agilent Technologies chromatograph 6890N (Agilent Technologies, Palo Alto, CA, USA) equipped with a flame ionization detector (FID), a splitless injector and a polar INNOWAX 30 M silica capillary column (0.25 mm i.d. & 0.25 µm film thickness). The temperature of the injector and detector were 220 °C and 275 °C, respectively. Helium was used as a carrier gas with a flow rate of 1.5 ml/min. Peaks were identified by comparison of their retention times with FAMES standards (SUPELCO). The sequences of fatty acids were ranged according to their chromatographic retention times and the values are given as percentages of the total fatty acids methyl esters.

Thiobarbituric acid reactive substance (TBARS)

The TBARS was determined according to the AOCS (1998) method. This procedure allows the direct determination of TBARS in oils and fats without preliminary isolation of secondary oxidation products; it is applicable to animal and vegetable fats and oils. Oil sample (50 to 200mg) dissolved in 1-butanol mixed with 0.2% TBA in 1-butanol were incubated 2 hours in a 95 °C water bath and cooled for 10 min under tap water (TBARS reaction). The absorbance was measured at 532 nm against a corresponding blank (a reaction with all the reagents and treatments except the oil extract). The standard curve was determined by the TBARS reaction of a series of aliquots (0.1 to 1 ml) of 0.2 mM TMP (1,1,3,3-tetramethoxypropane) prepared in 1-butanol. The results were expressed as mg malonaldehyde/kg of oil.

Total volatile bases (TVB-N)

The TVB-N was determined by flow injection analysis (Ruiz-Capillas & Horner 1999).

Trimethylamine (TMA-N)

The TMA-N was determined by flow injection analysis (Sadok *et al.* 1996).

pH

The pH was measured using a pH-meter at

room temperature on homogenates in water in a ratio 1:10 (w/v) (AOAC 1995).

Antioxidant activity of thyme

The Antioxidant activity was conducted as described by (Osawa & Namiki 1985). A sample of powdered thyme (1.3 mg) was dissolved in 10 ml of 50 mM phosphate buffer (pH=7), and was added to a solution of 0.13 ml of linoleic acid and 10 ml of 99.5 % ethanol. The total volume was then adjusted to 25 ml with distilled water. The mixture was incubated in a 50 ml assay tube with a screw cap at 40 ± 1° C in a dark room and the degree of oxidation was evaluated by measuring the ferric thiocyanate values according to the method of Mitsuta *et al.* (1966). A total of 100 µl of the oxidised linoleic acid solution was added to 4.7ml of 75 % ethanol, 0.1 ml of 30 % ammonium thiocyanate, and 0.1 ml of 0.02 M ferrous chloride solution in 3.5 % HCl. After 3 min stirring, the absorbance was measured at 500 nm. α-Tocopherol was used as reference and distilled water as control. The antioxidative capacity of inhibiting the peroxide formation in linoleic acid system was expressed as follows:

$$\text{Inhibition (\%)} = [1 - (\text{Absorbance of sample} / \text{Absorbance of control})] \times 100$$

DPPH assay I (%)

The free radical-scavenging activity was determined by the DPPH test using the method of Tepe *et al.* (2005). This test aims to measure the capacity of the extracts to scavenge the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) formed in solution by donation of hydrogen atom or an electron. If the extracts have the capacity to scavenge the DPPH free radical the initial blue/purple solution will change to a yellow colour due to the formation of diphenylpicrylhydrazine. This reaction is used as a measure of the ability of the extracts, or any other antioxidant, such as BHT, to scavenge any free radical.

To 5 ml of a methanol solution of 2,2'-diphenylpicrylhydrazyl (DPPH) 0.002% in methanol, 50 µl of essential oil or extract solutions were added and the mixture was incubated at room temperature for 30 min. The absorbance was measured at 517 nm against a corresponding blank.

Inhibition percentage of free radical DPPH was calculated in the following way:

$$I (\%) = (A_{\text{blanc}} - A_{\text{sample}} / A_{\text{blanc}}) \times 100$$

where A_{blanc} is the absorbance of the control reaction (a reaction with all the reagents except the test extract), and A_{sample} is the absorbance of the test extract. Tests were carried out in quadruplet and the extract concentration providing 50% inhibition (IC_{50}) was obtained by plotting extract solution concentration versus inhibition percentage.

Statistical analysis

Statistical analysis was performed using SPSS software, version 10.0.5. The comparison of flesh quality indicators during storage were tested using Duncan's test (95% confidence interval) with one-way ANOVA. Data are expressed as mean \pm standard error; n values are shown in each table and figure.

Results and Discussion

Biochemical analysis

Table I shows the changes of proximate composition of both tuna fillets lots during storage. The lipid and protein contents found in this study in fresh tuna were 10.49 and 16.21 g/100g, respectively. Mourente *et al.* (2001) reported for Atlantic female northern bluefin tuna (*Thunnus thynnus thynnus* L.) a low fat content ranging from 0.82 to 1.55 g/100g fresh white muscle. Lower lipid levels, have been reported also for *Thunnus alalunga* (0.88 ± 0.64 g lipid /100g fresh muscle) and *Euthynnus lineatus* (1 ± 0.3 g lipid /100g fresh muscle) (Ben-Gigirey *et al.* 1999, Manzano *et al.* 2000). The high tuna lipid level found in this study may be related to season. Generally, lipid content varies within species (1.46 to 5.77 g/100g) and is affected by the catching season (1.2 to 18.4 g/100g) (Bandarra *et al.* 1997, Osman *et al.* 2001).

According to Duncan's test, no significant differences ($p > 0.05$) were found for moisture, ash, protein and lipid between all fillets lots during the storage period. Similarly, carbohydrate content decreased slightly at the end of storage but remained no-significant for $p > 0.01$. These findings are in accordance with those reported by Chomnawang *et al.* (2007).

Fatty acids

Fatty acids composition of control and treated tuna fillets during refrigerated storage are presented in Tables II and III. Polyunsaturated fatty acids (PUFAs) constitute the majority of the fatty acids pool, followed by saturated (SFAs) and monounsaturated fatty acids (MUFAs). Within these groups, the major fatty acids were palmitic

acid (16:0), stearic acid (18:0) and oleic acid 18:1 (n-9 & n-7). The total polyenes content was 41.32%, with eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) being the prominent polyunsaturated fatty acids.

Significant differences ($p < 0.05$) in fatty acid profiles (SFAs, MUFAs and PUFAs) were observed in control lot before and after storage. SFAs and MUFAs percents increased from 30.66 to 34.16 and from 20.33 to 21.32 respectively, PUFAs levels remain unchanged during the three first days (from 41.32 % to 41.28 %), thereafter, a significant decrease ($p < 0.05$) was showed in day 6 (39.89 %) and in day 12 (38.74 %).

In treated lot, PUFAs levels remain significantly constant from day 0 (41.32 %) to day 15 (41.05 %), thereafter, a significant decrease was showed at the end of storage (day 18) to reach 39.38 % (Fig. 1). The decrease of PUFAs percentage may indicate the oxidation of these unsaturated fatty acids during storage. Such changes were not observed in thyme treated lot. The uses of natural antioxidant in fish fillets has induced the stability of fatty acids profiles without altering their composition and minimize such auto-oxidation. These results are in accordance with those reported by Serdaroglu & Felekoglu (2005).

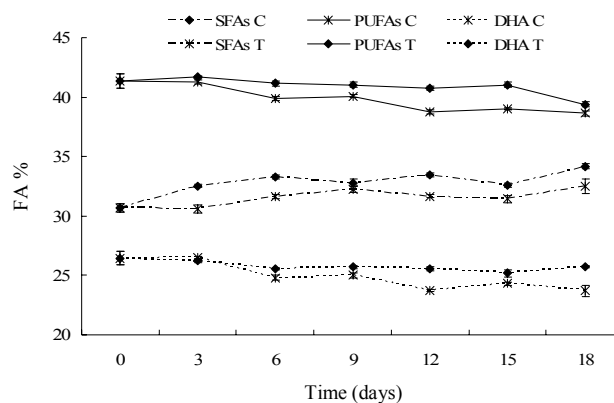


Figure 1. Changes in PUFAs, SFAs and DHA values of control and thyme treated tuna fillets during chilled storage. Bars indicate standard deviations from quadruplicate determinations. FA: fatty acid; PUFAs: polyunsaturated fatty acids; SFAs: saturated fatty acids; DHA: docosahexaenoic acid; C: control lot; T: treated lot.

Due to their high degree of unsaturation, EPA and DHA are readily oxidized. Such characteristic has suggested the use of the polyene index [(EPA + DHA)/16:0] and PUFAs/SFAs to evaluate oxidative deterioration of polyunsaturated fatty acid in fish lipids (Wada & Fang 1992). In this study, the polyene index and PUFAs/SFAs values in control samples decreased from 1.70 % to 1.52 % and from 1.35 % to 1.20 % following 15 days of

storage, respectively. No changes were observed in polyene index and PUFAs/SFAs values for thyme treated lot. It has been reported that sardines treated with rosemary extract and onion juice, retained ratio of [EPA + DHA/16:0] statically constant during storage (Serdaroglu & Felekoglu 2005).

TBARS

Several studies showed that TBARS values may be used as indicator of seafood lipid quality, whether fish was ice stored, chilled or frozen (Vareltzis *et al.* 1988). In this study, fresh tuna TBARS level was 0.34 mg malonaldehyde/kg (Fig. 2), such levels remained constant up to 6 days of chilled storage, with no significant differences between control and thyme treated lots. The observed stability of the product to lipid oxidation could be attributed to the chilling condition and to the O₂ barrier properties of the polyethylene bags used in the packages. Thereafter a significant increase was observed in both lots with higher levels in control lot ($p < 0.05$) toward the end of the storage (> 9 days of storage). The increase in TBARS indicated formation of secondary lipid oxidation products such as aldehydes and others volatiles compounds (Kolakowska 2002).

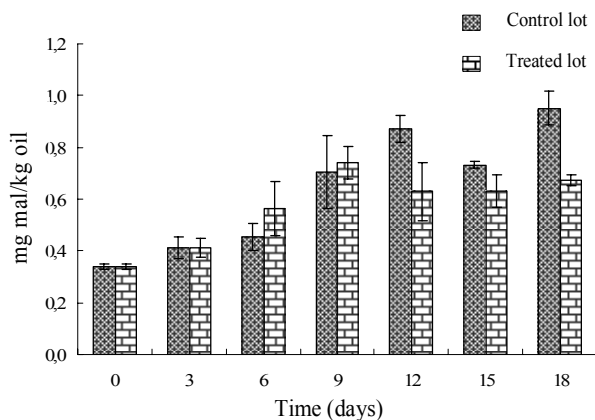


Figure 2. Changes in TBARS values of control and thyme treated tuna fillets during chilled storage. Bars indicate standard deviations from quadruplicate determinations. mal: malonaldehyde; TBARS: thiobarbituric acid reactive substance.

These findings are in accordance with the antioxidative effect of thyme *Thymus vulgaris* observed by others authors (Justesen & Knuthsen 2001, Sachetti *et al.* 2005).

Mazorra-Manzano *et al.* (2000) reported for black-skipjack (*Euthynnus lineatus*) muscle an increase ($p < 0.05$) in TBARS values from an initial value of 10 mg malonaldehyde/ kg sample to a final value of 52 mg malonaldehyde/kg after 24 days of iced storage. Ryder *et al.* (1984) reported for

mackerel (*Trachurus novaezelandie*) 8.3 mg malonaldehyde/100g after 7 days of ice storage and correlated this TBA level with off-odors and flavours for cooked fish as well as rancidity odors in gills. Chaijan *et al.* (2006) reported also that after 15 days of iced storage, TBARS value in sardine muscle increased by 97% (620 mg malonaldehyde/kg sample) when compared with that found in fresh muscle. In this study, TBARS of all examined tuna samples did not exceed 1 mg malonaldehyde/kg oil with a measured maximum value in control tuna sample at 18 days of chilled storage.

Antioxydant activity

Many natural antioxidants are plant phenolics found in every part of the plant, including the fruit, seeds and leaves (Kim *et al.* 1997, Shahidi 2000, Sachetti *et al.* 2005). The major constituent of Thyme *Thymus vulgaris* is Luteolin, to which are attributed many of the antioxidant properties (Justesen & Knuthsen 2001). The antioxidant activity may occur via various mechanisms such as the inhibitory effect on lipid peroxidation and by scavenging the radicals.

In our data, the ethanol thyme extract exhibit good radical scavenging activities ($IC_{50} = 18.6 \pm 0.5 \mu\text{g/ml}$) and it was significantly higher ($p < 0.05$) to that of standard butylatedhydroxytoluene. Differently, Mata *et al.* (2007) have reported a low radical scavenging activities for wild thyme *Thymus serpyllum* ($IC_{50} = 13.2 \pm 0.3 \mu\text{g/ml}$) and a high radical scavenging for *Mentha spicata* and *Rosmarinus officinalis* ($IC_{50} = 65.2 \pm 0.1 \mu\text{g/ml}$ and $36 \pm 0.1 \mu\text{g/ml}$ respectively).

The relative antioxidant inhibition for the powdered thyme was calculated against that of α -Tocopherol. The inhibition level of thyme was 78.07% and it is significantly ($p < 0.05$) lower than α -Tocopherol (83.58%). Plant extracts are complex mixtures and reports of antioxidant activities evaluated by different tests are not always concordant (Trouilla *et al.* 2003, Sachetti *et al.* 2005, Tepe *et al.* 2005, Mata *et al.* 2007).

TVB-N and TMA-N

Table IV shows the results of chemical analysis of control and thyme treated tuna fillets during chilled storage. The initial TVB-N level was 11.69 mg/100g, which is noticeably lower than levels found in other fresh tuna such as *Euthynnus lineatus* and *Thunnus alalunga* (25.5 and 22.89 mg/100g TVB-N respectively) (Ben-Gigirey *et al.*

Table I. Proximate composition of control and thyme treated tuna fillets during chilled storage. Means (n = 4) with the same letter within columns are not significantly different (p > 0.05).

Storage time (days)	Control lot					Thyme treated lot				
	Moisture (g/100g)	Ash (g/100g)	Lipids (g/100g)	Carbohydrates (mg/100g)	Proteins (g/100g)	Moisture (g/100g)	Ash (g/100g)	Lipids (g/100g)	Carbohydrates (mg/100g)	Proteins (g/100g)
0	70.60 ± 0.85a	1.22 ± 0.06a	10.49 ± 0.87a	80.45 ± 2.82ab	16.21 ± 0.89 a	70.60 ± 1.71a	1.26 ± 0.05a	10.49 ± 0.87a	80.45 ± 2.82ab	16.21 ± 0.89a
3	70.07 ± 0.58a	1.28 ± 0.09a	9.93 ± 0.53a	81.84 ± 2.74ab	15.35 ± 1.37a	69.26 ± 1.48a	1.21 ± 0.08a	11.16 ± 0.55a	67.06 ± 12.06a	14.84 ± 1.17a
6	69.10 ± 0.40a	1.36 ± 0.09a	10.39 ± 0.53a	80.76 ± 7.29ab	16.29 ± 1.21a	68.85 ± 0.34a	1.29 ± 0.08a	11.07 ± 0.63a	72.26 ± 7.40ab	15.74 ± 0.96a
9	69.48 ± 1.30a	1.27 ± 0.05a	9.85 ± 0.45a	82.92 ± 2.94ab	16.74 ± 0.94a	68.95 ± 0.13a	1.26 ± 0.07a	10.71 ± 1.18a	73.13 ± 7.49ab	16.06 ± 1.28a
12	69.80 ± 0.40a	1.30 ± 0.09a	10.38 ± 1.57a	80.52 ± 5.22ab	14.73 ± 1.16a	68.76 ± 0.13a	1.25 ± 0.08a	11.12 ± 0.74a	74.38 ± 5.84ab	15.46 ± 0.85a
15	69.58 ± 0.42a	1.26 ± 0.07a	10.94 ± 1.75a	82.60 ± 1.98a	15.43 ± 1.28a	70.64 ± 0.31a	1.34 ± 0.06a	11.14 ± 1.55a	84.09 ± 2.49b	16.83 ± 1.31a
18	69.43 ± 0.35a	1.20 ± 0.08a	10.59 ± 1.46a	75.39 ± 9.94b	15.49 ± 1.25a	69.66 ± 0.04a	1.26 ± 0.09a	11.94 ± 1.38a	74.42 ± 7.01ab	15.82 ± 0.69a

Table II. Change in fatty acids composition of control tuna fillet during chilled storage. Means (n = 4) with the same letter within rows are not significantly different (p > 0.05). The values do not represent 100% because minor fatty acids are not reported. Others SFAs: 15:0 ; 17:0 ; 20:0. Others MUFAs: 14:1 ; 15:1 ; 17:1 ; 20:1 n-9. Others PUFAs: 16:2; 20:2; 20:3.

Control	0 days	3 days	6 days	9 days	12 days	15 days	18 days
Fatty acids							
14:00	3.24 ± 0.12a	3.35 ± 0.03ab	3.68 ± 0.03d	3.60 ± 0.07cd	3.79 ± 0.02d	3.48 ± 0.05d	3.79 ± 0.05bc
16:00	19.94 ± 0.20a	20.93 ± 0.09bc	21.44 ± 0.13d	21.09 ± 0.20bcd	21.36 ± 0.13cd	21.56 ± 0.14b	20.68 ± 0.13d
18:00	5.60 ± 0.04a	6.28 ± 0.04e	6.11 ± 0.04cd	6.04 ± 0.01bc	6.16 ± 0.02d	7.00 ± 0.02b	6.01 ± 0.02f
others SFAs	1.89	1.98	2.06	2.05	2.12	2.11	2.08
SFAs	30.66 ± 0.36a	32.54 ± 0.13b	33.29 ± 0.19cd	32.78 ± 0.29bcd	33.44 ± 0.17d	32.58 ± 0.19bc	34.16 ± 0.22e
16:1 n-7	3.75 ± 0.12a	3.72 ± 0.02a	4.06 ± 0.04bcd	4.01 ± 0.05bc	4.20 ± 0.03d	3.94 ± 0.03cd	4.16 ± 0.03b
18:1 n-9	12.66 ± 0.26a	12.63 ± 0.04a	12.84 ± 0.11ab	12.95 ± 0.03ab	13.32 ± 0.06c	13.13 ± 0.07bc	13.13 ± 0.06bc
18:1 n-7	2.17 ± 0.05a	2.18 ± 0.00a	2.25 ± 0.02b	2.27 ± 0.00bc	2.32 ± 0.01c	2.31 ± 0.01bc	2.29 ± 0.01bc
others MUFAs	1.75	1.99	1.83	1.82	1.81	1.94	1.81
MUFAs	20.33 ± 0.37a	20.52 ± 0.06ab	20.98 ± 0.12bc	21.05 ± 0.08c	21.74 ± 0.09d	21.38 ± 0.09cd	21.32 ± 0.11cd
18:2 n-6	1.60 ± 0.04a	1.63 ± 0.01a	1.68 ± 0.01b	1.68 ± 0.00b	1.72 ± 0.01b	1.69 ± 0.01b	1.73 ± 0.01b
18:3 n-3	1.56 ± 0.05a	1.58 ± 0.00a	1.70 ± 0.01bc	1.69 ± 0.01b	1.73 ± 0.01bc	1.58 ± 0.01c	1.76 ± 0.01a
20:4 n-3	1.17 ± 0.03a	1.17 ± 0.02a	1.24 ± 0.01bc	1.21 ± 0.00b	1.23 ± 0.00bc	1.15 ± 0.00c	1.26 ± 0.01a
20:5 n-3	7.52 ± 0.11a	7.56 ± 0.03a	7.67 ± 0.03ab	7.68 ± 0.01ab	7.61 ± 0.03ab	7.14 ± 0.02b	7.76 ± 0.06c
22:5 n-3	1.48 ± 0.06a	1.26 ± 0.02b	1.26 ± 0.02b	1.31 ± 0.01b	1.28 ± 0.00b	1.26 ± 0.01b	1.33 ± 0.01b
22:6 n-3	26.45 ± 0.55a	26.50 ± 0.08a	24.81 ± 0.19b	25.02 ± 0.21b	23.73 ± 0.10c	24.30 ± 0.17c	23.70 ± 0.46bc
others PUFAs	1.54	1.59	1.53	1.48	1.44	1.53	1.48
PUFAs	41.32 ± 0.60a	41.28 ± 0.07a	39.89 ± 0.21bc	40.08 ± 0.21b	38.74 ± 0.14d	39.03 ± 0.18d	38.64 ± 0.20d
EPA/DHA	0.28 ± 0.00a	0.29 ± 0.00a	0.31 ± 0.00b	0.31 ± 0.00b	0.32 ± 0.00c	0.33 ± 0.00c	0.29 ± 0.00d
PUFAs/SFAs	1.35 ± 0.01a	1.27 ± 0.00b	1.20 ± 0.01c	1.22 ± 0.02c	1.16 ± 0.01d	1.20 ± 0.01c	1.13 ± 0.01d

Table III. Change in fatty acids composition of thyme treated tuna fillet during chilled storage. Means (n = 4) with the same letter within rows are not significantly different (p > 0.05). The values do not represent 100% because minor fatty acids are not reported. Others SFAs: 15:0 ; 17:0 ; 20:0. Others MUFAs: 14:1 ; 15:1 ; 17:1 ; 20:1 n-9. Others PUFAs: 16:2; 20:2; 20:3.

Thyme treated lot	0 days	3 days	6 days	9 days	12 days	15 days	18 days
Fatty acids							
14:00	3.24 ± 0.12ab	3.37 ± 0.08abc	3.54 ± 0.03bc	3.46 ± 0.05bc	3.39 ± 0.01abc	3.18 ± 0.13a	3.28 ± 0.06abc
16:00	19.94 ± 0.20ab	19.55 ± 0.24a	20.24 ± 0.10abc	20.58 ± 0.14bc	20.19 ± 0.08abc	20.80 ± 0.42c	20.19 ± 0.17abc
18:00	5.60 ± 0.04a	5.85 ± 0.02ab	5.87 ± 0.03ab	6.20 ± 0.03bc	6.07 ± 0.03b	6.51 ± 0.29c	6.11 ± 0.02b
others SFAs	1.89	1.81	2	2.02	2.01	1.99	1.83
SFAs	30.66 ± 0.36ab	30.57 ± 0.37a	31.65 ± 0.15bc	32.24 ± 0.23c	31.65 ± 0.12bc	31.42 ± 0.28c	32.49 ± 0.58abc
16:1 n-7	3.75 ± 0.12a	3.83 ± 0.04ab	4.00 ± 0.02b	3.94 ± 0.04ab	3.94 ± 0.01ab	3.79 ± 0.14ab	3.90 ± 0.03ab
18:1 n-9	12.66 ± 0.26a	12.86 ± 0.03a	12.81 ± 0.04a	12.92 ± 0.05a	12.93 ± 0.04a	12.88 ± 0.25a	12.79 ± 0.04a
18:1 n-7	2.17 ± 0.05a	2.25 ± 0.01ab	2.26 ± 0.01ab	2.26 ± 0.01ab	2.29 ± 0.01b	2.20 ± 0.06ab	2.25 ± 0.00ab
others MUFAs	1.75	1.78	1.78	1.72	1.86	2.14	1.88
MUFAs	20.33 ± 0.37a	20.71 ± 0.04ab	20.85 ± 0.05ab	20.94 ± 0.09ab	21.03 ± 0.07b	20.81 ± 0.07b	21.02 ± 0.35ab
18:2 n-6	1.60 ± 0.04a	1.69 ± 0.01bc	1.72 ± 0.01c	1.70 ± 0.01bc	1.68 ± 0.00abc	1.62 ± 0.06ab	1.68 ± 0.01abc
18:3 n-3	1.56 ± 0.05a	1.72 ± 0.00c	1.77 ± 0.00c	1.69 ± 0.01bc	1.68 ± 0.01abc	1.58 ± 0.09ab	1.68 ± 0.01abc
20:4 n-3	1.17 ± 0.03a	1.27 ± 0.01a	1.29 ± 0.01a	1.23 ± 0.01a	1.24 ± 0.01a	0.88 ± 0.24b	1.25 ± 0.01a
20:5 n-3	7.52 ± 0.11ab	7.88 ± 0.04c	8.01 ± 0.03c	7.82 ± 0.03bc	7.80 ± 0.03bc	5.73 ± 1.51a	7.79 ± 0.04bc
22:5 n-3	1.48 ± 0.06a	1.42 ± 0.01ab	1.39 ± 0.02ab	1.36 ± 0.02bc	1.34 ± 0.01bc	1.25 ± 0.07c	1.37 ± 0.02ab
22:6 n-3	26.45 ± 0.55a	26.23 ± 0.14ab	25.54 ± 0.10bc	25.70 ± 0.11abc	25.54 ± 0.17bc	25.20 ± 0.31c	25.76 ± 0.09abc
others PUFAs	1.54	1.5	1.44	1.55	1.54	1.63	1.51
PUFAs	41.32 ± 0.60a	41.73 ± 0.16a	41.15 ± 0.15a	41.04 ± 0.12a	40.74 ± 0.19a	41.05 ± 0.08a	39.38 ± 0.24b
EPA/DHA	0.28 ± 0.00a	0.30 ± 0.00b	0.31 ± 0.00b	0.30 ± 0.00b	0.31 ± 0.00b	0.30 ± 0.00a	0.29 ± 0.01b
PUFAs/SFAs	1.35 ± 0.01ab	1.37 ± 0.02a	1.30 ± 0.01bc	1.27 ± 0.01c	1.29 ± 0.01c	1.31 ± 0.01d	1.21 ± 0.03bc

Table IV. Change in total volatile bases, trimethylamine and pH of control and thyme treated tuna fillets during chilled storage. Means (n = 4) with the same letter within columns are not significantly different (p > 0.05).

Storage time (days)	Control lot			Thyme treated lot		
	TVB-N	TMA-N	pH	TVB-N	TMA-N	pH
0	11.69 ± 0.85a	0.56 ± 0.16a	6.27 ± 0.06a	11.69 ± 0.85a	0.56 ± 0.16a	6.27 ± 0.06a
3	12.76 ± 0.58a	0.58 ± 0.10a	6.21 ± 0.13a	12.95 ± 0.37a	0.68 ± 0.07a	6.25 ± 0.10a
6	12.37 ± 0.40a	0.47 ± 0.09a	6.19 ± 0.11a	12.79 ± 0.59a	0.72 ± 0.11a	6.22 ± 0.13a
9	11.85 ± 1.30a	0.47 ± 0.15a	6.24 ± 0.07a	13.04 ± 0.56a	0.66 ± 0.07a	6.17 ± 0.12a
12	12.11 ± 0.40a	0.49 ± 0.11a	6.31 ± 0.09a	13.29 ± 0.95a	0.61 ± 0.11a	6.32 ± 0.07a
15	12.24 ± 0.42a	0.65 ± 0.14a	6.34 ± 0.08a	15.08 ± 1.05b	0.74 ± 0.15a	6.26 ± 0.08a
18	12.78 ± 0.35a	0.55 ± 0.13a	6.26 ± 0.11a	15.50 ± 0.80b	0.74 ± 0.15a	6.29 ± 0.09a

1999, Manzano *et al.* 2000). The initial TVB-N levels found in this study indicated that the fish were of high quality. Throughout the refrigerated storage period, the TVB-N content in tuna-control lot showed no significant changes. In the thyme-treated lot, the TVB-N showed a slight increase towards the end of the storage period, although it was statistically significant. In all lots, TVB-N levels remained significantly lower than the current rejection limit of 30-35 mg/100g (EEC 1995).

The initial TMA-N level in tuna flesh was 0.56 mg/100g, showing no change ($p > 0.05$) with levels far below the limit of acceptability of 10-15 mgTMA/100g (Sikorski *et al.* 1989) in all lots during the period of storage. It appears from this study that TVB-N and TMA indices do not provide a good indication of tuna *T. thynnus* freshness or spoilage, as these volatile bases levels were quite constant throughout the storage period although TVB-N parameter is considered as a legal index.

pH

The initial pH value of flesh tuna fillets was 6.27. Such level remained statically constant ($p > 0.05$) in both lots during the period of storage (Table IV). These results are in disagreement with those reported by Ruiz-Capillas & Moral (2001), who found an increase in pH of black-skipjack *Euthynnus lineatus* muscle after 24 days of ice-storage. It is well known that pH changes during refrigerated storage differ according to fish species and other factors such as the degree of microorganism contamination and storage condition (Ben-Gigirey *et al.* 1999).

Conclusion

Post-mortem biochemical changes indicated that tuna could be stored up to 18 days of chilled storage at 0°C. Tuna lipids were susceptible to oxidation during chilled storage but thyme-treatment minimized such auto-oxidation. These observations were consistently confirmed by the TBARS and polyunsaturated fatty acids data. Moreover, powdered thyme *Thymus vulgaris* can be used as an easily accessible source of natural antioxidants in commercial food products as they have better consumer acceptance.

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References

- Akhtar, P., Gray, J. I., Booren, A. M. & Garling, D. L. 1998. Effect of dietary components and surface application of oleoresin rosemary in lipid stability of rainbow trout (*Oncorhynchus mykiss*) muscle during refrigerated and frozen storage. **Journal of Food Lipids**, 5: 43-45.
- AOAC. 1990. Official methods of analysis (15th ed). **Association of Official Analytical Chemists**. Washington, DC. 1280 p.
- AOAC. 1995. Official methods of analysis. **Association of Official Analytical Chemists**. Washington, DC. 2000 p.
- AOCS. 1998. **American Oil Chemists' Society**. Official method Cd 19-90. 2-Thiobarbituric acid value. Direct method. In Official Methods and Recommended Practices of the American Oil Chemists' Society, 5th ed. (D. Firestone, ed.) AOCS. Champaign, III.
- Bandarra, N. M., Batista, I., Nunes, M. L., Empis J. M. & Christie W. W. 1997. Seasonal change in lipid composition of sardine (*Sardina pilchardus*). **Journal of Food Science**, 62: 40-42.
- Ben-Gigirey, B., Baptista De Sousa, J. M. V., Villa, T. G. & Barros-Velazquez, J. 1999. Chemical Changes and Visual Appearance of Albacore Tuna as Related to Frozen Storage. **Journal of Food Science**, 64: 20-24.
- Boran, G., Karacam, H. & Boran, M. 2006. Changes in the quality of fish oils due to storage temperature and time. **Food Chemistry**, 98: 693-698.
- Chaieb, K., Hajlaoui, H., Zmanter, T., Kahla-Nakbi, A., Rouabhia, M., Mahdouani, K. & Bakhrouf, A. 2007. The chemical composition and biological activity of clove essential oil, *Eugenia caryophyllata* (*Syzygium aromaticum* L. Myrtaceae). Short review. **Phytotherapy Research**, 21: 501-506.
- Chaijan, M., Benjakul, S., Visessanguan, W. & Faustman, C. 2006. Changes of lipids in sardine (*Sardinella gibbosa*) muscle during iced storage. **Food Chemistry**, 99: 83-91.
- Chomnawang, C., Nantachai, K., Yongsawatdigul, J., Thawornchinsombut, S. & Tungkawachara, S. 2007. Chemical and biochemical changes of hybrid catfish fillet stored at 4 °C and its gel properties. **Food Chemistry**, 103: 420-427.
- Donelli, J. K. & Robinson, D. S. 1995. Free radicals in foods. **Free Radical Research**, 22: 147-176.
- Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers,

- P. A. & Smith, F. 1956. Colorimetric method for determination of sugars and related substances. **Analytical Chemistry**, 28, 350-356.
- EEC, European Economic Commission. 1995. Determinación de la concentración de bases nitrogenadas volátiles (NBVT) en pescados y productos de la pesca procedimiento de referencia. **Diario Oficial de las Comunidades Europeas**, N°L 97, Directive 149: 84-7.
- Folch, J., Lees, M. & Stanley, G. H. S. 1957. A simple method for the isolation and purification of total lipids from animal tissue. **Journal of Biological Chemistry**, 226: 497-509.
- Frankel, E. N. 1993. In search of better methods to evaluate natural antioxidants and oxidative stability in food lipids. **Trends in Food Science and Technology**, 4: 220-225.
- Hollman, P. C. H., Hertog, M. G. L. & Katan, M. B. 1996. Analysis and health effects of flavonoids. **Food Chemistry**, 57: 43-46.
- Justesen, U. & Knuthsen, P. 2001. Composition of flavonoids in fresh herbs and calculation of flavonoid intake by use of herbs in traditional Danish dishes. **Food Chemistry**, 73: 245-250.
- Karpinska, M., Borowski, J. & Danowska-Oziewicz, M. 2001. The use of natural antioxidants in ready-to-serve food. **Food Chemistry**, 72: 5-9.
- Kim, B., Kim, J., Kim, H. & Heo, M. 1997. Biological screening of 100 plants for cosmetic use. II. Antioxidative activity and free radical scavenging activity. **International Journal of Cosmetic Sciences**, 19: 299-307.
- Kolakowska, A. 2002. Lipid oxidation in food systems. In Z. Sikorski & A. Kolakowska (Eds.), **Chemical and functional properties of food lipids** (pp. 133-165). London, UK: CRC Press.
- Lacroix, M., Smoragiewicz, W., Pazdernik, L., Koné, M. I. & Krzystyniak, K. 1997. Prevention of lipid radiolysis by natural antioxidants from rosemary (*Rosmarinus officinalis* L.) and thyme (*Thymus vulgaris* L.). **Food Research International**, 30: 457-462.
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. 1951. Protein measurement with the folin phenol reagent. **Journal of Biological Chemistry**, 193: 265-275.
- Manzano, M. A. M., Pacheco-Aguilar, R., Díaz-Rojas, E. I. & Lugo-Sánchez, M. E. 2000. Postmortem Changes in Black Skipjack Muscle During Storage in Ice. **Journal of Food Science**, 65: 774-779.
- Mata, A. T., Proença, C., Ferreira, A. R., Serralheiro, M. L. M., Nogueira, J.M.F., Araujo, M.E.M. 2007. Antioxidant and antiacetylcholinesterase activities of five plants used as Portuguese food spices. **Food chemistry**, 103: 778-786.
- Mazorra-Manzano, M. A., Pacheco-aguilar, R., Díaz-Rojas, E. I. & Lugo-Sánchez, M. E. 2000. Postmortem changes in black skipjack muscle during storage in ice. **Journal of Food Science**, 65: 774-779.
- Metcalfe, L. D., Schimitz, A. A. & Pelka J. R. 1966. Rapid preparation of fatty acids esters from lipids for gas chromatographic analysis. **Annexe Chemistry**, 38: 524-535.
- Mitsuta, H., Yasumoto, K. & Iwami, K. 1996. Antioxidative action of indole compounds during the autoxidation of linoleic acid. **Eiyoto Shokuryo**, 19: 210-214.
- Mourente, G., Megina, C., & Díaz-Salvago, E. 2001. Lipids in female northern bluefin tuna (*Thunnus thynnus thynnus* L.) during sexual maturation. **Fish Physiology and Biochemistry**, 24: 351-363.
- Osawa, T. & Namiki, M. 1985. Natural antioxidant isolated from eucalyptus leaf waxes. **Journal of Agriculture and Food Chemistry**, 33: 777-780.
- Osman, H., Suriah, A. R. & Law, E. C. 2001. Fatty acid composition and cholesterol content of selected marine fish in Malaysian waters. **Food Chemistry**, 73: 55-60.
- Powell, C. J., Connelly, J. C., Jones, S. M., Grasso, P. & Bridges, J. W. 1986. Epatic responses to the administration of high doses of BHT to the rat: Their relevance to epatocarcinogenicity. **Food and Chemical Toxicology**, 24: 1131-1143.
- Ruiz-Capillas, C. & Horner, W. F. A. 1999. Determination of the trimethylamine and total volatile basic nitrogen in flesh fish by flow injection analysis. **Journal of the Science of Food and Agriculture**, 79: 1982-1986.
- Ruiz-Capillas, C. & Moral, A. 2001. Correlation between biochemical and sensory quality indices in hake stored in ice. **Food Research International**, 34: 441-447.
- Ryder, J. M, Buisson, D. H., Scott, D. N., Fletcher, G. C. 1984. Storage of New Zealand Jack mackerel (*Trachurus novaezelandiae*) in ice:

- chemical, microbiological and sensory assessment. **Journal of Food Science**, 49: 1453-1456.
- Sachetti, G., Maietti, S., Muzzoli, M., Scaglianti, M., Manfredini, S., Radice, M. & Renato-Bruni, R. 2005. Comparative evaluation of 11 essential oils of different origin as functional antioxidants, antiradicals and antimicrobials in foods. **Food Chemistry**, 91: 621-632.
- Sadok, S., Uglow, R. F. & Haswell, S. J. 1996. Determination of trimethylamine in fish by flow injection analysis. **Analytica Chimica Acta**, 321: 69-74.
- Serdaroglu, M. & Felekoglu, E. 2005. Effects of using rosemary extract and onion juice on oxidative stability of sardine (*Sardina pilchardus*) mince. **Journal of Food Quality**, 28: 109-120.
- Shahidi, F. 2000. Antioxidants in food and food antioxidants. **Nahrung**, 44: 158-163.
- Sikorski, Z. E., Kolakowska, A. & Burt, J. R. 1989. **Post harvest biochemical and microbial changes. Seafood: resources, nutritional composition and preservation**. Boca Raton, Florida: CRC Press Inc.
- Tepe, B., Daferera, D., Sokmen, A., Sokmen, M. & Polissiou, M. 2005. Antimicrobial and antioxidant activities of the essential oil and various extracts of *Salvia tomentosa* Miller (Lamiaceae). **Food Chemistry**, 90: 333-340.
- Thompson, D. C. & Trush, M. A. 1986. The toxicological implications of the interaction of butylated hydroxytoluene with other antioxidants and phenolic chemicals. **Food Chemistry and Toxicology**, 24: 1189-1195.
- Trouilla, P., Calliste, C. A., Allais, D. P., Simon, A., Marfak, A. & Delage, C. 2003. Antioxidant, anti-inflammatory and antiproliferative properties of sixteen water plant extracts used in the limousine countryside as herbal teas. **Food Chemistry**, 80: 399-407.
- Vareltzis, K., Zetou, F. & Tsiaras, I. 1988. Textural deterioration of chub mackerel (*Scomber japonicus* collias) and smooth hound (*Mustelus mustelus* L.) in frozen storage in relation to chemical parameter. **Lebensmittel-Wissenschaft Und-Technologie**, 21: 206-211.
- Wada, S. & Fang, X. 1992. The synergistic antioxidant effect of rosemary extract and tocopherol in sardine oil model system and frozen crushed fish meat. **Journal of Food Processing and Preservation**, 16: 263-274.

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