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# 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-treaded 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, antixidante, 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 ($BF_3$/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/mn. 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:

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

*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 diphenyl-picrylhydrazine. 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 blanc.

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

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

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 ± 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 buefin 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 decease ($p < 0.05$) 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).

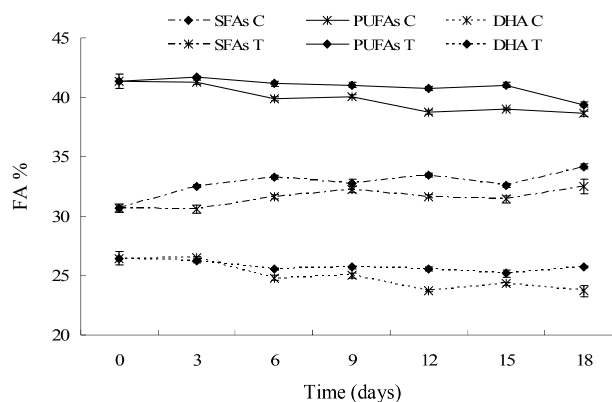


**Figure 1**. Changes in PUFAs, SFAs and DHA values of control and thyme treated tuna fillets during chilled storage. Bars indicate standard deviations from quadruplicate determinations. FA: fatty acid; PUFAs: polyunsaturated fatty acids; SFAs: saturated fatty acids; DHA: docosahexaenoic acid; C: control 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 $O_2$ 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).

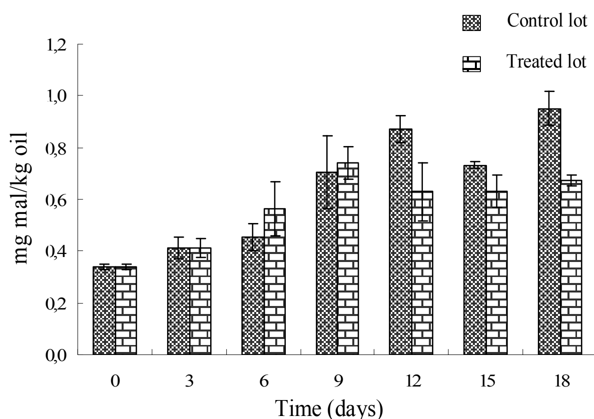


**Figure 2.** Changes in TBARS values of control and thyme treated tuna fillets during chilled storage. Bars indicate standard deviations from quadruplicate determinations. mal: malonaldehyde; TBARS: thiobarbituric acid reactive substance.

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 naturals 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 wich 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 ($IC_{50} = 18.6 \pm 0.5$ µg/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* ($IC_{50} = 13.2 \pm 0.3$ µg/ml) and a high radical scavenging for *Mentha spicata* and *Rosmarinus officinalis* ($IC_{50} = 65.2 \pm 0.1$ µg/ml and $36 \pm 0.1$ µg/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).

| | Control lot | | | | | Thyme treated lot | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Storage time (days) | 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 ($p > 0.05$).

| | Control lot | | | Thyme treated lot | | |
|---|---|---|---|---|---|---|
| Storage time (days) | 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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