***Original Research Article***

# *Thymus vulgaris* L. essential oil: effect on fatty acids and proteins oxidation of little tuna (*Euthynnus alletteratus*) minced during refrigerated storage.


## ABSTRACT

*People are uncomfortable having their food chemically treated to boost its shelf life. Thus, Tunisian Thymus vulgaris essential oil (TVEO) were investigated in parallel with its capacity to limit fatty acids and proteins oxidation in tuna (Euthynnus alletteratus) minced during refrigeration storage (4°C) has been conducted. Also, time-related survival, at 4°C, of S. typhimurium experimentally inoculated ($10^3$ CFU/g) in minced tuna and treated with MIC and MBC of TVEO was studied. Our results revealed that for tuna preservation, the measured concentrations of PV, TBARS and TVB-N during different storage periods showed a good efficiency of this EO (3%) in limiting lipids and proteins oxidation of tuna flesh. Also, we observed increase in all lots was significantly different (P< 0.05) and lots treated with TVEO were the most conserved comparing to the BHT. Overall outcomes suggest that TVEO use for tuna conservation could represent a promising strategy to improve the qualitative characteristics as well as the safety of seafood.*



***Keywords:*** *Thyme essential oil; minced tuna; refrigerated storage; lipids alteration; protein oxidation.*


## 1. INTRODUCTION

Health promoting properties, recently linked to aromatic and medicinal plants, as well as to their Essential oils (EOs), are attracting more and more attention due to their diverse applications in food technology, pharmaceuticals and phytomedicine [1-6]. Their potent antimicrobial and antioxidant properties provide the opportunity to be a good a safer alternative to synthetics [7-12]. Currently, a major problem in the food industry is to ensure proper preservation of food. However, oxidation and microorganism's contamination are limiting factors. Indeed, the microbiological quality of a food constitutes one of the essential bases of its ability to satisfy consumer safety. Contamination of food with bacteria and fungi contributes to its deterioration and to the decrease of its sensory, nutritional and health characteristics. In addition, oxidative degradation leads to loss of vitamins, deterioration of lipids and proteins, deterioration of flavour, decrease in nutritional value and even sometimes to the appearance of toxic substances [13]. Nowadays, all over the world, stored food products suffer serious damage, leading to considerable economic losses and especially health risk. Each year worldwide, unsafe food causes 600 million cases of foodborne diseases and 420 000 deaths. 30% of foodborne deaths occur among children under 5 years of age [14]. In the United States, we estimate that foodborne diseases cause approximately 76 million illnesses, 325,000 hospitalizations, and 5,000 deaths each year. 9.4 million cases were caused by several pathogenic microorganisms [15].

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**Comment [Ma2]:** Full meaning first before abbreviation

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**Comment [Ma4]:** reference

Seafood is a major global food commodity and an important part of a healthy diet. It is an important source of proteins. In 2016, 47% of seafood was already supplied by aquaculture and around 17% of animal protein consumed worldwide came from aquatic animals (fish, crustaceans and molluscs) [16]. Seafood participates in global health risks. In fact, worldwide, seafood is one of the main foods at risk because it is responsible for a large number of food-borne illnesses [17]. For example, in Europe, seafood was suspected to cause around 11% of collective foodborne illnesses reported in 2018 [18]. Foodborne illnesses are generally infectious or toxic in nature and are caused by bacteria, viruses, parasites or chemicals that enter the body through contaminated food. The risks of contamination exist throughout the food chain, from primary production to the preparation of products for consumption [19]. Nowadays, we cannot neglect an eventual contamination by Corona virus which can be found in seawater via the discharge of wastewater and consequently the contamination of seafood. Bacteria causing infectious food diseases can be classified into two categories: native bacteria, naturally present in marine and estuarine environments, such as *Vibrio* spp., and introduced bacteria, the occurrence of which is accidental. The latter are *Enterobacteriaceae* and their presence is due to the contamination of the aquatic environment by faecal pollution or contamination occurring during the preparation or processing of seafood products [19]. Several studies on seafood have shown high levels of contamination by several bacteria such as *Aeromonas hydrophila, Vibrio parahaemolyticus, Vibrio vulnificus, Staphylococcus* aureus, *Listeria monocytogenes*, *Pseudomonas*, *Salmonella* spp. [20,21]. A study conducted by the Food and Drug Administration (FDA) in U.S. has shown that for fish and shellfish, an overall incidence of *Salmonella* is~~of~~ 1.3% in national seafood and 7.2% for imported ones [19]. In the European Union, *Salmonella* genus takes the second position among bacterial genera responsible for gastrointestinal human's epidemics [22].

Seafood is fragile food products that require good prevention and risk control methods during all food chain stages, from collection to consumption. However, to deal with the problems of oxidation and contamination, a variety of synthetic chemicals as food preservatives has been considered. These have shown unwanted side effects over time. However, several synthetic preservatives have been limited in several countries, due to their toxicological effects, including carcinogenicity [23,24].

Therefore, the aim of this study is to investigate the inhibition effect of TVEO on fatty acids and proteins alteration of little tuna (*Euthynnus alletteratus*) stored at 4°C- for~~during~~ 16 days.

## 2. MATERIAL AND METHODS

### 2.1. *Thymus vulgaris* essential oil

In this experiment we used fresh TVEO,~~ the extraction of which was carried out in our research unit~~. This oil chemotype is carvacrol (75%).

**Comment [Ma5]:** Describe how the oil was extracted

### 2.2. Fish Sampling and Processing

Little tuna (*Euthynnus alletteratus*) was caught in the Northern area of the Tunisian Sea. The fishes were transported on ice to the laboratory where they were eviscerated, headed and filleted. Each piece was immersed in boiling water for 1 min, in order to reduce microorganisms attached to the fillet surface. The pieces of little tuna prepared as above were minced in a sterile grinder. In order to test TVEO effect on limitation of lipid and protein alteration in tuna samples, three lots were prepared: one was left as a control lot, the second

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was added the BHT and the third was added a 3% of TVEO. All lots were then vacuum packed in polyethylene bags and stored at +4°C for 16 days.

Peroxide value (PV), thiobarbituric acid reactive substance (TBARS) and total volatile bases nitrogen (TVB-N) were determined at days 0, 4, 8, 12 and 16.

### 2.3. Determination of peroxide value

The peroxide value (PV), expressed as mEeq of active $O_2$ per kg of lipid, was determined using the method described by Guran *et al*. [25]. The free fatty acid (FFA) content of the lipid was determined volumetrically using aqueous sodium hydroxide (0.1 N) and phenolphthalein indicator (1% ethanol).

### 2.4. Determination of Thiobarbituric acid reactive substance (TBARS)

The TBARS was determined according to the AOCS [26] method and based on the same protocol as done by Hajlaoui *et al.* [27].

### 2.5. Determination of total volatile bases nitrogen (TVB-N)

Total volatile basic nitrogen (TVB-N) was determined according to the method described by Varlik *et al.* [28].

### 2.6. Statistical analysis

All the experiments were conducted in triplicate and average values were calculated using the SPSS 26.0 statistics package for Windows. The differences in mean were calculated using the Duncan's multiple-range tests for means with 95% confidence limit ($P \leq 0.05$).

## 3. RESULTS AND DISCUSSION

### 3.1. Effect of the EO addition on the lipids and proteins oxidation

#### 3.1.1. Effect on the fat alteration

*** Determination of peroxide value (PV)**

Hydroperoxides are among the first products formed during lipid oxidation reactions. These are unstable molecules rapidly broken down into secondary products such as aldehydes, ketones, alcohols, etc. They were assayed directly on methanolic extracts of tuna flesh in order to characterize the degree of primary lipids oxidation. Evolution of hydroperoxides concentrations measured on different samples, stored at 4°C for 16 days without (negative control) and with TVEO and BHT (positive control), arewere presented in **fig. 1**.

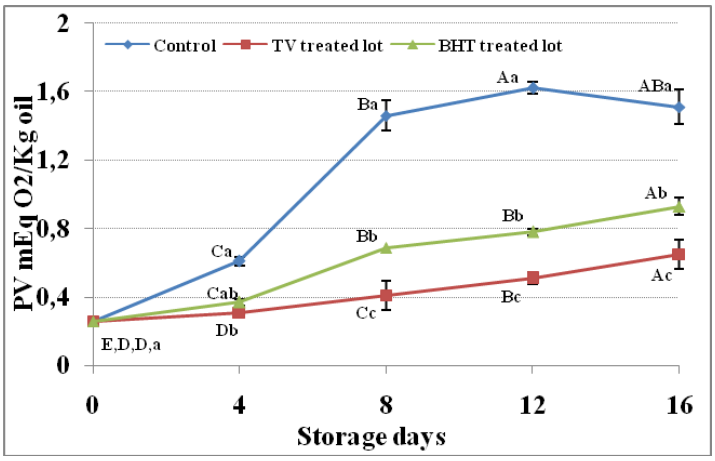


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**Fig. 1. Evolution of the peroxide index in the tuna flesh (PV in meq $O_2$ /kg of lipid) during chilled storage (+4°C) during 16 days in absence (C = Control) and in the presence TVEO and BHT. Each value represents the average of 3 repetitions.**

These results showed a significant increase ($P \leq 0.05$) in PV over storage time of all samples. But this development was dependent on added antioxidant. However, negative control batch PVs were systematically higher than those of stored TVEO and BHT batches for different storage periods, which indicate significant lipids oxidation reaching its maximum after 12 days of storage. Concerning batches treated by TVEO and BHT and for all storage periods, PVs were lower than control ones. This result revealed a significant ($P \leq 0.05$) limitation of fatty acids oxidation in flesh tuna. In fact, TVEO was more effective than BHT in this limitation. However, the PVs of batches treated with TVEO are significantly ($P \leq 0.05$) lower than the PVs of batches treated with BHT for all storage periods. After 16 days of storage, PVs measured were 1.51±0.1; 0.93±0.08 and 0.65±0.05 meq $O_2$/kg of lipid respectively for untreated, treated with BHT and treated with TVEO lots.

**Comment [Ma6]:** This statement does not indicate significant lipid oxidation reaching its maximum after 2 days

This parameter was also measured in other study. Guran *et al*. [25] showed~~ing~~ that for untreated fish patties and treated with Thyme, Clover, and Rosemary EO, PV values fluctuated significantly ($P < 0.05$) between 0.51 and 2.69 mmole active oxygen/kg lipids during storage. A lower PV was observed for samples treated with Rosemary indicating that this EO prevents more fish patties from undergoing oxidation.

*** Thiobarbituric acid reactive substance (TBARS)**

Results in fig. 2 showed TBARS concentrations evolution measured on untreated and treated with TVEO and BHT lots stored at +4°C for 16 days. During the first 4 days of storage, statistical analysis showed that TBARS values were the same ($P >0.05$) for all stored samples which values were 0.67±0.17 mg MDA/kg lipid. This observed stability of lipid oxidation could be attributed to cooling conditions and to properties of polyethylene bags used in packaging which represent a good barrier against $O_2$ [29,30]. After 4 days of storage, TBARS values increased in the untreated and BHT treated batches. While TVEO treated batch, TBARS values increased significantly ($P<0.05$) only after 8 days of storage. This explains the great ability of TVEO to inhibit lipid~~s~~ oxidation. This capacity is significantly ($P <0.05$) greater than the synthetic antioxidant (BHT). After 16 days of storage, TBARS values of untreated batch, increased 6 times compared to day zero to reach the value of 4.19±0.06 mg MDA/kg of lipid. While TBARS values were tripled in BHT treated batches and only doubled in the TVEO treated batches. This important reducing effect of TBARS may be

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attributed to the strong antioxidant properties of the TVEO. Similar results with EOs (thyme, clove and rosemary) were reported by Guran *et al.* [25] indicating that TBARS of fish patties ~~were~~ ranged from 0.33 to 4.04 mg MDA/kg after 16 days of storage with rosemary EO has the most capacity. In addition, Kenar *et al.* [31] revealed that TBARS value in control was 0.58 mg MDA/kg in vacuum packed sardines while a little fluctuation was mentioned when it was treated with rosemary extract (10g/l) at 3°C reaching 0.84 mg MDA/kg after 20 days of storage. On the other hand, TVEO treatment seems to be more efficient to decrease TBARS value in flesh fish than treatment with powdered thyme. However, the study of Selmi and Sadok [30] revealed that no significant differences between control and fresh tuna (*Thunnus thynnus*) sprinkled by powdered thyme lots.

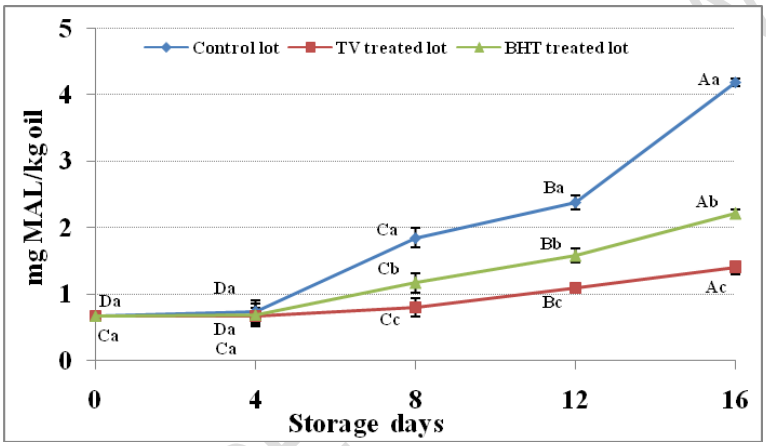


**Fig. 2. Evolution of TBARS concentrations in the tuna flesh (in Mg MDA/kg of lipid) during storage with +4°C during 16 days in absence (C=Control) and in the presence of TVEO and of BHT. Each value represents the average of 3 repetitions.**

Several studies showed also that temperatures variation of meat and fish storage were an important factor in lipid oxidation. For example, Rong *et al.* [32] compared TBARS value of Atlantic mackerel stored in 4 and 0°C which they ranged from 0.23±0.04 mg/kg to 2.60±0.08 mg/kg and to 2.16±0.12 mg/kg after five days of storage at 4°C and 0°C, respectively. This later was confirmed by others studies like Wang *et al.* [33] which reported that 0° C was more effective than 4°C to inhibiting lipid oxidation of salmon.

### 3.1.2. Effect on total volatile basic nitrogen (TVB-N)

TVB-N mainly consists of amines such as methylamine, dimethylamine, and trimethylamine, they were the most widely used index for fish freshness assessment [20]. **Fig. 3** showed TVB-N values evolution of untreated and treated (TVEO and BHT) of little tuna lots during storage at 4°C. The initial TVB-N level was 8.23±0.05 mg/100g, which is noticeably lower than levels found in other fresh tuna such as *Thunnus thynnus, Euthynnus lineatus* and *Thunnus alalunga* (11.69; 25.5 and 22.89 mg/100g respectively) [18]. Kinetics of TVB-N values as a function of storage periods showed a significant difference ($P$ <0.05) between the three lots. In fact, the lowest TVB-N is recorded in the treated lots by TVEO,

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followed by BHT treated lots. While Control lots showed high values indicating spoilage of tuna flesh. After 16 days of storage, the TVB-N values were 27.05±1; 22.88±0.1 and 14.44±0.6 mg/100g respectively for control, BHT and TVEO lots. Otero *et al.* [34] suggested that the increase in TVB-N value in fish during cold storage is mainly due to the alkalinity substances produced by the protein degradation by enzymatic reactions and microbial activity.

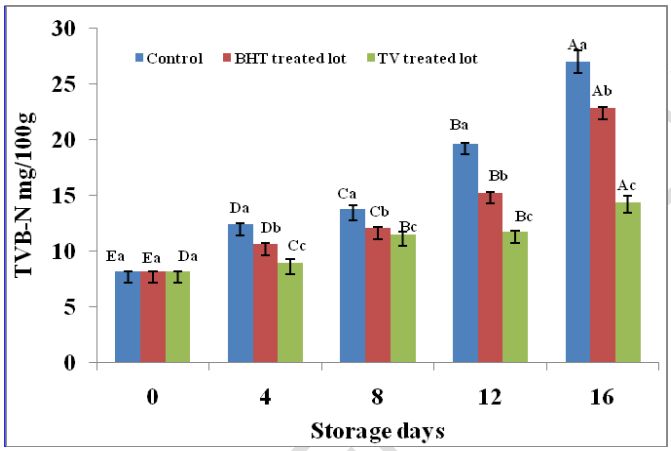


**Fig. 3. Changes in TVB-N (total volatile basic nitrogen) values during storage (16 days) at +4°C of little tuna no treated (C=control), treated with TVEO and treated with BHT. Each value represents the average of 3 repetitions.**

**Comment [Ma7]:** At day 0 they are alnost the same but they are carrying different suoerscript.
There should be a footnote to describe the statistical difference

These results showed that TVEO has a great capacity on limiting flesh tuna proteins deterioration. This ability is explained by the antioxidant and antimicrobial power that this oil has. This limitation of TVB-N is mainly due to the high percentage of carvacrol. Despite this preservation, there is some alteration which is due to several factors initiating the oxidation such as; initiation by activated forms of oxygen, initiation by metals and other environmental factors like; temperature, pH, and oxygen partial pressure. Noting that oxidation is a very complicated phenomenon resulting in the interaction between different compounds in fish flesh. However, after fish death, lipoxygenases would be released by the skin and generate hydroperoxides of lipids within the muscles. These hydroperoxides participate in the initiation and propagation of auto-oxidation [35]. During storage, hydroperoxides and secondary products of lipid oxidation interact with proteins and amino acids. Free radicals produced by the lipid oxidation can promote protein oxidation to form carbonyl groups and change the secondary structure [32,26]. These interactions have a significant impact on degradation of functional, sensory and nutritional properties of foods [27].

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## 4. CONCLUSION

In summary, TVEO is highly effective in ensuring the preservation of tuna during storage at + 4 ° C for 16 days. However, the addition of 3% of this EO showed a limitation of primary and

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secondary oxidation of lipids as well as proteins deterioration of tuna flesh. PV, TBARS and TVB-N concentrations measured after 16 days of storage are 0.65±0.05 meq $O_2$/kg of lipid, 1.4 ± 0.05 mg MDA/kg of lipid and 22.78±0.1 mg/100g, respectively. These values indicate good preservation of these physicochemical parameters. During the different periods of storage, the use of TVEO in preservation is more effective than the synthetic antioxidant (BHT). These results promote the use of TVEO as a preservative for seafood.

**Comment [Ma9]:** Values should not be in conclusion

## CONSENT

It is not applicable.

## ETHICAL APPROVAL

It is not applicable.

## COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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