



## Influence of Using Chitosan and Thyme during Cooling Storage of Sea Bass Fish

A. M. Abouel-Yazeed

Food Science Dept., Fac. Agric. (Saba Basha), Alex. Univ. Alexandria, Egypt.



CrossMark

**T**HIS study aimed to apply and study the effect of natural preservatives thyme oil and chitosan on the storage of sea bass at  $4\pm 1^\circ\text{C}$  for 16 days as well as its quality parameters. Samples of the study were fillets of fish coated with thyme oil (Thy) or chitosan (Ch) and mix of thyme oil and chitosan (Thy+ Ch) while C (control without any addition). Analysis conducted every four days during storage period. Results showed the most indicated amino acid were the glutamic addition to aspartic acid. High contents of indispensable amino acid, lysine besides leucine, were also detected. Furthermore, fatty acid profile presented that the palmitic acid (23.57%) was the primary saturated fatty acid while the major polyunsaturated fatty acids included linoleic acid, docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA).

The chemical analyses of the results at zero time presented the pH also Total volatile base nitrogen (TVB-N) addition, Thiobarbituric acid (TBA) as well as free fatty acid were 6.48, 8.59 mg/100 g, 0.11 mg malonaldehyde/kg and 1.23 % (expressed as % of oleic acid), respectively. The initial Psychrotrophic and *Enterobacteriaceae* counts were 2.21 log<sub>10</sub> cfu/g and 1.59 log<sub>10</sub> cfu/g, respectively. During storage, gradual increase was observed in each sample and significant variations were found among samples ( $P < 0.05$ ).

The results of sensory analysis revealed that the storage period of fish fillets was four days for control sample, 12 and 16 days in the treated with (Thy), (Ch) and (Thy+ Ch), respectively ( $P < 0.05$ ). Lastly, addition of (Thy+ Ch) indicated a positive result of the storage period of fish products. However, the addition of the mixture of (Thy+ Ch) can retard unfavorable chemical changes, also lipid oxidation, increase sensory qualities and prolong the storage of the product throughout cold storage at  $4^\circ\text{C} \pm 1^\circ\text{C}$  for 16 days.

**Keywords:** Sea bass (*Dicentrarchus labrax*), Natural preservatives, Shelf - life.

### Introduction

Seafood is a respected portion of human food, because of its content of polyunsaturated fatty acids (PUFA), besides protein (Pezeshk et al., 2015, Kazeml and Rezael 2015, FAO 2018 and Houessou et al., 2019). PUFA of these products have developed attention because of the protection of human cardiovascular (Ozogul et al., 2006, Campos et al., 2019 and Hematyar et al., 2019). However, synthetic antioxidant have to be widely

applied due to fat oxidation (Benjakul et al., 2005 and Prato et al., 2019).

Otherwise, with developing concerns about the safety of synthetic antioxidants, the usage of natural antioxidants was recommended (Banerjee, 2006 and Gammone et al., 2019). Sea bass (*Dicentrarchus labrax*) is unique of the finest preferred for many people (Alasalvar et al., 2002). Cooling is one of the most well – known preservation technique, which may not sufficient to

\*Correspondence to : amrfarouk01@hotmail.com

Received:19/7/2019; accepted:3/11/2019

DOI: 10.21608/EJFS.2019.15009.1013

©2019 National Information and Documentation Centre (NIDOC)

preventing fat deterioration, off-aroma otherwise microbial increase (Gomez-Guillen and Montero, 2007). Different studies deal with changes in chemical as well as the sensory characteristics of bass fish during cooling have been published (Poli et al., 2001 and Taliadourou et al., 2003).

In plenty of condition, there is another necessity for increasing seafood quality through extracts from botanical resources which proved the best efficacious usages to increase food storage (Appendini and Hotchkiss, 2002, Hanusova et al., 2009, Erkan et al., 2011, Falguera et al., 2011 and Sousa et al., 2019). (Appendini and Hotchkiss, 2002, Hanusova et al., 2009 and Sousa et al., 2019).

Herbal oils are achieved broad attention in manufacturing to possible as safety agents, as they are Generally Recognized as Safe (GRAS) (Gutierrez et al., 2009 and Roomiani et al., 2019).

Phenolic type, play a role in inhibiting Gram-positive or Gram-negative microbes (Mangena and Muyima, 1999, Marino et al., 2001 and Skandamis et al., 2002). Submersion of thyme with oregano oil were improved progress meat-based products storage (Karabagias et al., 2011). Thyme essential oil (TEOs) contains many phenolic compounds which showed antimicrobial as well as antioxidant activity (Karabagias et al., 2011).

TEOs showed a strong inhibitory effect on *Staphylococcus aureus* or *Bacillus subtilis* also, *Escherichia coli* as well as *Salmonella enteritidis* (Solomakos et al., 2008) and against food borne pathogens besides spoilage microbes (Gutierrez et al., 2008). So, thyme is able to consider as best replace for synthetic additives in food.

Treatment by TEO with cooling has shown benefit technique to expand the storage foods (Holley and Patel 2005 and Chouliara et al., 2007). Chitosan (Ch) is a natural polysaccharide manufactured by chitin deacetylation (Govaris et al., 2010 and Pandharipande et al., 2018).

In acidic status, Ch enter to the cell wall synthesis and break - down, it happens thereafter bacterial death (Duan et al., 2010). Ch has been wide employed in food preservation (Feng et al., 2004 and Khodanazary et al., 2019). Lopez-Caballero et al. (2005) stated layer mix of chitosan with gelatin applied restrained influence on the gram-negative of fish patties.

To achieve an antibacterial effect, chitosan is based on the attraction between its positively charged particles and the negative charges of microbiological cell membrane (Erkan et al., 2015) in addition to its role as a barrier for oxygen move (Jeon et al., 2002 and Shyu et al., 2019).

Gunlu and Koyun (2013) reported storage fish fillets treated by chitosan was extended about twenty days under cool ( $4 \pm 1$  °C). Ojagh et al. (2010) illustrated treatment by chitosan besides cinnamon better-quality trout fish storage (sixteen days). Consequently, the target of the present study was to analyze the amino acids besides, the fatty acid profile of sea bass (*Dicentrarchus labrax*) affected during storage. Moreover, the purpose of the study is knowing the influence of the thyme essential oils, chitosan-based edible film and combined with of them with the chemical also, microbiological and sensory characteristic of sea bass fillets through storage at  $4 \pm 1$  °C.

## Material and Methods

### Materials

#### Fish sample

Fresh sea bass (*Dicentrarchus labrax*) was obtained during October 2018, (10 kg) with an average weight and length: 300–330 g and 25–30 cm. Fish was obtained from Al-Anfoshy, Alexandria Governorate, Egypt. Fish was placed (in ice tanks) in ice with a fish/ice proportion of 1:3 (w/w) and transported to the Department of Food Sciences, Faculty of Agriculture, Saba Bacha, Alexandria University.

Chitosan in the form of fine crystals was purchased from a local company, EL-Helw Co., Cairo. Obtained from shrimp waste.

#### Thyme oil

Thyme essential oil was purchased from a local company; (food grade, extra pure *Tylenchorhynchus vulgaris* France, El-Kamal, Co., Egypt). 2.1.4.

#### Chemicals and reagents

Solvents, chemicals, and reagents were obtained from El-Gomhouria Company, Alexandria, Egypt, and Sigma–Aldrich (Germany). All chemicals and reagents used were of analytical grade.

### Methods

#### Fish samples preparation

Fresh sea bass (*Dicentrarchus labrax*) was immediately washed, headed, eviscerated as described by Etemadian et al. (2012) with stainless steel knife, washed again (cooled water)

and drained for 20 min on a sanitized stainless steel throughout cooled storage (Shi et al., 2014).

#### *Preparation of chitosan-based edible films*

For chitosan - based edible biofilms, commercial chitosan obtained from shrimp shells was used and edible films were prepared as described by (Gunlu and Koyun, 2013) and Tingting et al., (2013). Thyme oil, thyme essential oil; 0.2% (v/v) mixed with Polysorbate 0.5% to stabilize the emulsion, Giatrakou et al., (2013). The mix of chitosan and thyme oil solution was prepared with 1.5 % (w/v) chitosan and 0.2% (v/v) thyme oil in solution 1% (v/v) acetic acid and following the same procedure of chitosan preparation.

Fillets previously prepared were randomly divided into four samples treatment consisting of C (Control) sample (Not immersed in any solution), Thy (immersed in thyme oil solution), Ch (immersed in chitosan solution) and (Thy+ Ch) immersed in mix of thyme oil and chitosan solution). Fillets were immersed for 30 min in 1500 ml of the different solution and then the fish fillets were removed and allowed to drain for 30 min on a sanitized stainless steel throughout refrigerated storage. Samples were stored at  $4\pm 1^{\circ}\text{C}$  for subsequent quality assessment.

Chemical, microbiological and sensorial analyses were performed at 4-day intervals to determine the overall quality of fish.

#### *Chemical analyses*

##### *Gross chemical composition*

The gross chemical composition of the fish fillets was assessed; the moisture content and total ash were determined in an oven at  $103^{\circ}\text{C}$  and  $550^{\circ}\text{C}$ , respectively, until a constant weight. The crude protein was determined by micro Kjeldahl's method (A.O.A.C. 2002).

##### *Total lipid content*

Samples were minced using a mincer (Mincy meat mincer Rigamontr Art-125 made in Italy). Total lipids were extracted from minced samples according to Folch Method (Folch et al., 1957) using chloroform: methanol (2:1, v/v).

##### *Amino acid profile*

Amino acids were determined using Beckman Amino Acid Analyzer Model 119 CL, according to the method described by Moore (1958).

##### *Fatty acid profile*

Gas Chromatography (GC) Fatty acids

methyl esters (FAMES) were analyzed using (GC) (Hewlett Packard, Palo Alto, CA, USA) (HP 6890) and (FID) detector at  $250^{\circ}\text{C}$ , siloxane capillary column HP - 5 (30m x 0.32 mm I.D. x 0.25  $\mu\text{m}$  film thickness). under (0.8 m / min nitrogen gas flow), according to the method of (Radwan, 1978). A standard mixture of methyl esters was analyzed under identical condition prior to running the samples. The retention times of the unknown samples of methyl esters were compared with those of standard.

#### *Chemical analyses*

##### *Determination of pH measurement*

The pH measurement was achieved using pH-meter type JENCO (Micro pH-vision 6071), according to Masniyom, et al., (2005).

##### *Determination of total volatile base-nitrogen (TVB-N):*

TVB-N in samples was determined according to Parvaneh, (2007).

##### *Determination of thiobarbituric acid (TBA) value*

Thiobarbituric acid (TBA) was determined by colorimetric method according to Tarladgis *et al.*, (1960), and Kilinc *et al.*, (2007). The absorbance was measured at 538 nm (using Spectrophotometer T 80 UV/vis Spectrometer PG Instrument Lta) against a blank, which was 5 mL of distilled water with 5 mL TBA reagent. The absorbance (D) against the blank at 538 nm by 1 cm cell. TBA was expressed as mg malonaldehyde/kg sample. TBA No (as mg malonaldehyde/kg sample) = 7.8 D.

##### *Determination of free fatty acid (FFA)*

FFA analysis expressed as % oleic acid was done by the Egan *et al.*, (1981). Free fatty acid (FFA) content was determined by mixing 20 mL of the chloroform extract with an equal volume of neutral alcohol and titrating with 0.01N NaOH in the presence of phenol-phthalein indicator.

#### *Microbiological analysis*

##### *Preparation of samples*

Ten grams of fish tasters were homogenized in 90 ml sterile physiological saline 0.9% supplemented by 0.1% peptone. Decimal dilutions were then made in duplicate in a sterile physiological saline containing 0.1% peptone. Several dilutions were prepared to be used for counting psychrotrophic aerobic bacteria, *Enterobacteriaceae* group, Microbial counts were expressed as the logarithm of colony forming units per g ( $\log_{10}$  CFU/g).

#### *Psychrotrophic aerobic bacteria*

It was determined using plate count agar media (PCA, Oxoid C. M 325) and incubated for 5 days at 22°C (Vander et al., 2002).

#### *Enterobacteriaceae group count*

Counts were determined using plating appropriate dilutions of samples on violet red bile glucose agar (VRBGA, Oxoid, C. M. 485) appropriate sample dilution (1 ml) was pour plate on a VRBGA media, and incubated for 24 hr at 37°C according to Mossel et al. (1979).

#### *Colour and odour evaluation*

The sensory evaluation was used to evaluate the quality of fish, and the end of the shelf-life (all samples were stored at 4°C). It was reached when the average value of the samples were judged as unacceptable by the panelists. A score of 5 point hedonic scale was considered as the lowest limit of acceptability (Fan et al., 2009).

Five members participated in scoring all the tested quality attributes of fish. The five panelists were from the Department of Food Science, Faculty of Agriculture, Saba-Bacha, Alexandria University. Each group of samples were labeled and randomly selected. Panelists were asked to evaluate each batch of samples, presented in a randomized (Patsias, et al., 2006) order to assign scores for colour: (general appearance, surface slime) and odour (fishy, smell). Colour and odour evaluation was determined using a nine-point hedonic scale (9=like extremely; 8=like very much; 7=like moderately; 6=like slightly; 5=neither like nor dislike; 4=dislike slightly; 3=dislike moderately; 2=dislike very much; 1=dislike extremely).

#### *Statistical Analyses*

All experiments were carried out and data were presented as mean values  $\pm$  standard deviations (SD) and a probability value of ( $P < 0.05$ ) was considered significant. Analysis of variance (ANOVA) was performed and the mean comparisons were done by Duncan's multiple range tests, (1955). Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS for Windows, SPSS Inc., and Chicago, IL, USA).

## **Results and Discussion**

#### *Proximate chemical composition*

Chemical compositions of bass fish are presented in Table 1. Results obtained indicated that, the moisture in the bass fish fillets was (g/100g fish muscle)  $77.30 \pm .53\%$ , while the protein was  $18.85 \pm .34\%$ , Total lipid was 2.50

$\pm .29\%$  and ash was  $1.51 \pm .61\%$  which similar to the data obtained through (Grigorakis et al., 2005 and Ozogul et al., 2005). Alasalvar et al. (2002) stated that protein in fish varied depending on season.

Meanwhile, they revealed an opposite correlation between moisture and protein. Ozogul et al. (2005) detected lipid and moisture of hake fish (*Merluccius hubbsi*) fillets different due to relatively consist of fish food. Moreover, concerning to the reproduction of bass fish.

Such variations within the proximate analyses of fish is connected to nutrition, spawning, age, sexual, size and the different environmental conditions (Gonzalez-Fandos et al., 2005).

**TABLE 1. Proximate chemical analyses of bass fish fillets (on wet bases).**

Chemical constituents	g/100 g fish
Moisture	$77.30 \pm 0.53$
Crude protein	$18.85 \pm 0.34$
Total lipid	$2.50 \pm 0.29$
Ash	$1.51 \pm 0.61$

Values are mean  $\pm$  standard error from triplicate determinations.

**TABLE 2. Amino acid profile of bass fish fillets.**

Amino acids	(g/100g fish)
Leucine*	$6.73 \pm 0.32$
Isoleucine*	$4.52 \pm 0.87$
Threonine*	$3.98 \pm 0.95$
Valine*	$5.41 \pm 0.67$
Methionine*	$2.83 \pm 0.35$
Phenylalanine*	$3.97 \pm 0.79$
Lysine*	$9.58 \pm 0.31$
Histidine*	$2.75 \pm 0.64$
Arginine*	$5.53 \pm 0.35$
Aspartic acid	$9.73 \pm 0.28$
Serine	$5.89 \pm 0.54$
Glutamic acid	$9.97 \pm 0.89$
Glycine	$5.52 \pm 0.45$
Alanine	$6.63 \pm 0.81$
Proline	$5.54 \pm 0.76$
Tyrosine	$3.51 \pm 0.65$
E/NE	0.96

Mean values  $\pm$  Standard Deviation Values. *Note.* Tryptophan not determined. Essential amino acid for humans\*. Total essential amino acids, Ratio of essential / non-essential amino acids, E/NE.



*Amino acid analyses*

Amino acid profile of bass fish are presented in (Table 2). Results displayed that, the basic amino acids were glutamic, aspartic acid addition to lysine. The methionine, tyrosine along with histidine amounts, were less than the other of amino acids. This agrees with Ozden and Erkan (2008).

Fish are perfectly rich in lysine (Ozden and Erkan, 2008) and other described that the major amino acids in bass were aspartic and glutamic in addition to lysine. Physiologically, part of the protein stored in fish eggs during reproduction, which affected in the amino acids profile of fish (Ozyurt & Polat, 2006 and Ozden & Erkan, 2008). In the same respect, Wesselinova (2000) claimed that the quantities addition to varieties of amino acids were thoroughly affected by catching besides place as well. In the current search, the ratio of essential (E) / non-essential (NE) was found to be (0.96). The percentage of E/NE was decided as 0.71 for cod fish (*Gadusmorhua*) and 0.77 for sea bream (*Pagrus major*) by Jhaveri et al. (1984).

*Fatty acids profile*

The fatty acid profile of bass is presented in (Table 3). It consists of 35.67% saturated (SFA), 32.31% monounsaturated (MUFA) and 32.02% polyunsaturated (PUFA). The main fatty acids in bassfish fillets were palmitic acid ( $C_{16:0}$ ), oleic acid ( $C_{18:1}$ ) and eicosapentaenoic acid (EPA,  $C_{20:5}$   $\omega_3$ ) as well as docosahexaenoic acid (DHA,  $C_{22:6}$   $\omega_3$ ), in agreed to Alasalvar et al., (2002).

Stansby (1982), furthermore, detected palmitic acid with 20–50% of total fatty acid in several fish types. Major PUFA identified were linoleic acid, DHA and EPA. Similar results for bass were reported by Alasalvar et al. (2002) and Ozden & Erkan (2008). The proportion of  $\omega$ -3 to  $\omega$ -6 fatty acids was (1.91) which is similar for farmed bass of Greece and Spain (Fuentes et al., 2010). While, the proportion of  $\omega$ -3 to  $\omega$ -6 fatty acids for farmed and wild bass were recently stated as 2.88 and 3.02, respectively by Alasalvar et al. (2002).

*Chemical analysis**pH values of fish samples*

The pH of bass fish fillets are showed in Fig. 1. In the start of storage, pH value of bass fish fillets was found to be 6.48. Comparable results were stated by Kaya et al. (2006) for bonito fish. At the end of storage period, pH was 6.82. The sample coated with thyme oil (Thy) for 12 days have pH 6.95 and then discarded because unacceptable by the panelists. Meanwhile, sample Ch (coated with chitosan) for 12 days have pH 6.84. Thy+Ch coated sample stand for 16 days have pH 6.89.

A score of five- point hedonic scale was measured as the lowest end of acceptability (Fan et al., 2009). The pH of every treatments increased throughout storage, perhaps owing to the output of basic amines like trimethylamine as well as other volatile amines through spoilage of microbes (Ordonez et al., 2000). Statistically significant ( $P < 0.05$ ) variances were observed amongst all the samples for pH values according to the period of storage.

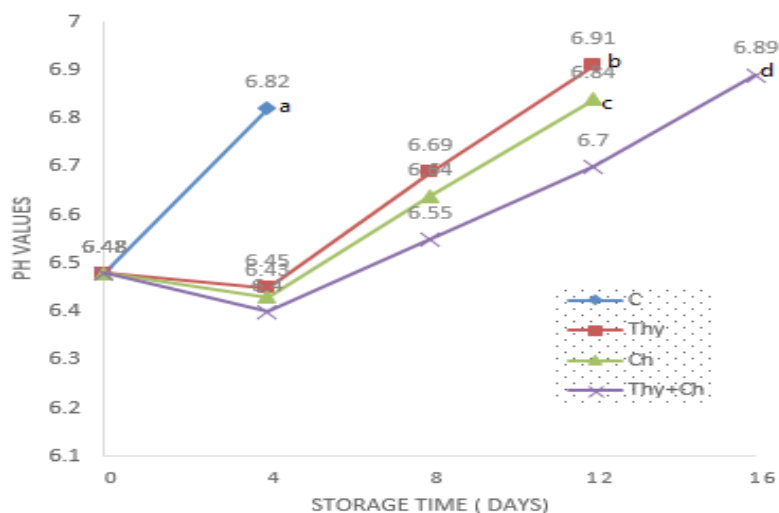
Chitosan solution's stability is poor above pH 7 due to precipitation that takes place in alkali pH range (Garci et al., 2010). Related with the control (pH 7.0), the treated samples revealed markedly lower pH.

**TABLE 3. Fatty acid profile of bass fish fillets. (% of total fatty acids).**

Fatty acids	(%)
$C_{14:0}$	1.64±0.36
$C_{15:0}$	1.53±0.86
$C_{16:0}$	23.57±0.34
$C_{17:0}$	1.93±0.73
$C_{18:0}$	6.15±0.22
$C_{21:0}$	0.85±0.98
$\Sigma$ SFA	35.67±0.77
$C_{14:1}$	0.35±0.42
$C_{16:1}$	5.34±0.32
$C_{17:1}$	1.72±0.73
$C_{18:1}$	21.89±0.84
$C_{20:1n9}$	1.59±0.23
$C_{22:1}$	1.42±0.64
$\Sigma$ MUFA	32.31±0.70
$C_{18:2}$	9.24±0.54
$C_{18:3}$	1.52±0.21
$C_{20:2}$	0.82±0.13
$C_{20:3}$	ND
$C_{20:4}$	0.94±0.68
$C_{20:5}$	6.61±0.79
$C_{22:6}$	12.89±0.46
$\Sigma$ PUFA	32.02±0.66
$\Sigma$ $\omega$ -3	21.02
$\Sigma$ $\omega$ -6	11.00
$\Sigma$ $\omega$ -3/ $\Sigma$ $\omega$ -6	1.91

Mean values ± Standard Deviation Values

ND = Not determined.



C: control without any addition. Thy: coated with thyme oil solution. Ch: coated with chitosan solution. Thy+ Ch: coated with mix of thyme oil and chitosan.

**Fig. 1. Changes in pH values of coated bass fish fillets with thyme oil solution, chitosan solution and their mixes during refrigerated storage at 4 °C for 16 days. Letters show significant differences between the samples at (p<0.05).**

#### *Total volatile base nitrogen (TVB-N) of fish samples*

TVB-N is an over-all expression which includes the quantity of trimethylamine or dimethyl-amine add ammonia, as well as other volatile basic nitrogenous compounds related to food spoilage (Huss, 1995). TVB-N that is produced by decomposition of protein owing to the action of microorganisms as well as enzymes is composed of ammonia and monoethylamine in addition dimethylamine, also, trimethylamine. TVB-N is usually used as an indicator of the quality of marine and aquatic products (Lee et al., 2007 and Kilinceker et al., 2009).

Level of 30 mg TVB-N/100 g of fish is considered as the suitable limit (Connell, 1990). The concentration of TVB-N in fresh fish is characteristically among 5 and 20 mg mg/100 g, while 30–35 mg/100 g flesh are usually described as the limit of suitability for iced-stored cold water fish (Huss, 1988 and Connell, 1995). The TVB-N values from various samples through storage are showed in Fig. 2.

After 4 days of storage, the TVB-N of control was 15.30 mg/100 g while the TVB-N for the Thy, Ch and (Thy+ Ch) treatments were 12.89, 11.20 and 10.93 mg/100 g, respectively. Thus, TVB-N of the control were higher than the other samples. After 8 days of storage TVB-N of (Thy+ Ch) were 21.15, 19.50 and 14.91 mg/100 respectively. That yet within the acceptable.

The samples treated by (Thy+ Ch) lower of the TVB-N values contrast with the treatment coated by Thyor, Ch only and control. The samples treated with (Thy+ Ch) lower due fast reduced microbial or lower ability of microorganisms for oxidative and de-amination of protein or together (Fan et al., 2008).

Jeon et al. (2002) reported that coating cod and herring fillets with soluble chitosan exhibited a decrease with 33–50% and 26–51%, respectively, in TVB-N after 12-day of storage. A rise in TVB-N could also, due to de-amination of free amino acids or oxidation of amines in addition to deterioration of nucleotides with autolytic enzymes and bacterial activity (Ocano-Higuera et al., 2011).

#### *Thiobarbituric acid (TBA) content of fish samples*

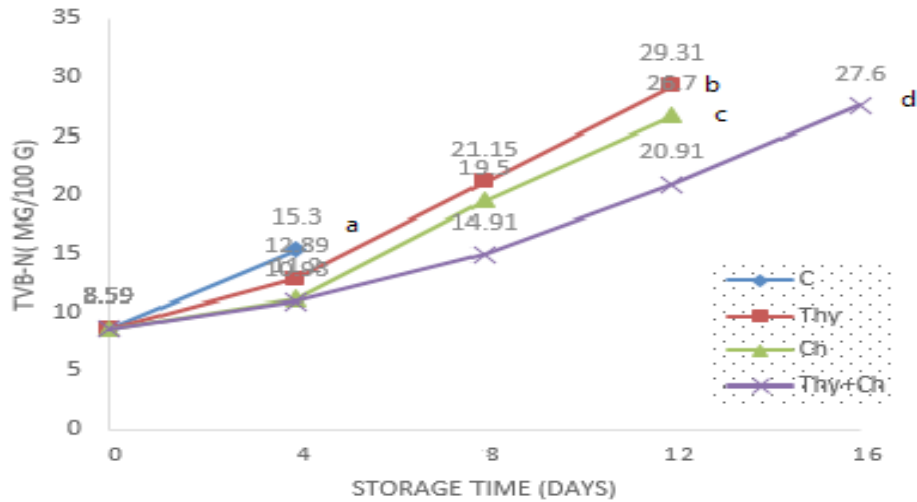
In accordance to Connell (1990), TBA values of 1-2 mg MDA / 1 kg of fish flesh are generally considered as the acceptable limit beyond that fish will usually progress a disagreeable. Changes of TBA during different treatments through storage are presented in Fig. 3.

The TBA of control was higher than the all treated samples. After 4 days, TBA for control sample was 1.1 mg MDA/kg. After 8 days of storage, the TBA of treatment by Thy up to 0.97 mg MDA/kg, that still suitable the acceptable range. Moreover, the second comprised treatment of Ch sample, TBA was 0.78 mg MDA/kg.

TBA for samples (Thy+ Ch) was 0.55 mg MDA/kg that was less than the other two samples groups. Furthermore, TBA values have been studied for meat preserved by oregano in addition to sage essential oil by (Fasseas et al., 2008). Results display Ch able to inhibit fat peroxidation, also, of thyme oil improved this effect.

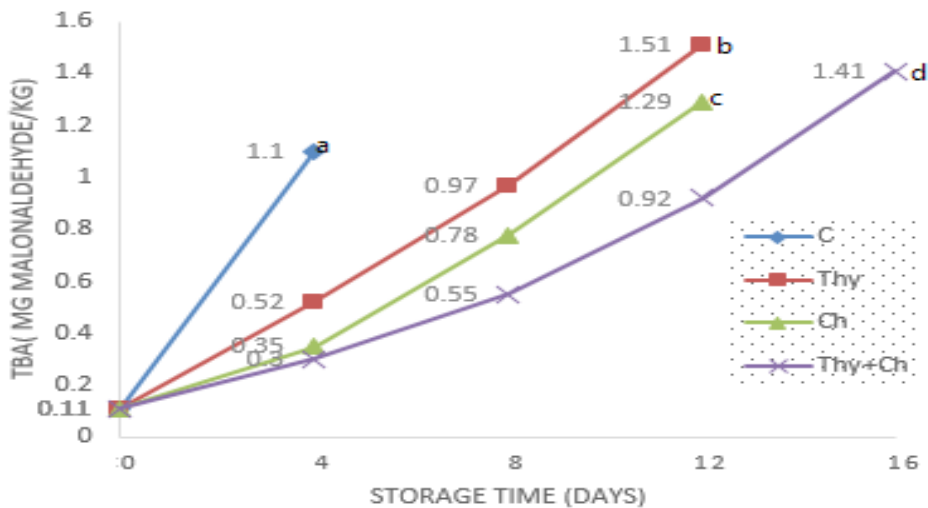
Coated sample with (Thy+ Ch) showed a

lower TBA values in comparison with the treated ones coated with Thy, Ch as well as the control. Adding of 0.1% thyme essential oil prolonged the product's storage according to Kykkidou et al., (2009), which is due to the strong antioxidant of the essential oil. Comparable, results using essential oils were described by Kenar et al. (2010), Ucak et al. (2011) and Emir Çoban et al. (2014).



C: control without any addition. Thy: coated with thyme oil solution. Ch: coated with chitosan solution. Thy+ Ch: coated with mix of thyme oil and chitosan.

Fig. 2. Changes in (TVB-N) values of coated bass fish fillets with thyme oil solution, chitosan solution and their mixes during refrigerated storage at 4 °C for 16 days. Letters show significant differences between the samples at (p<0.05).



C: control without any addition. Thy: coated with thyme oil solution. Ch: coated with chitosan solution. Thy+ Ch: coated with mix of thyme oil and chitosan.

Fig. 3. Changes in (TBA) values of coated bass fish fillets with thyme oil solution, chitosan solution and their mixes during refrigerated storage at 4 °C for 16 days. Letters show significant differences between the samples at (p<0.05).

#### Free Fatty Acid (FFA) of fish samples

In this research, FFA increased with a significant differences ( $P < 0.05$ ) among treated samples. FFA in the start of storage was of 1.23 % (expressed as % of oleic acid) (Fig. 4). On day 4, the FFA of the control was 4.67 % whereas the FFA for the Thy, Ch and (Thy+ Ch) samples treatments were 3.61, 3.11 and 2.85 %, respectively.

After 8 days of cooled storage, FFA of Thy, Ch and (Thy+ Ch) samples were 5.23, 4.95 and 3.67%, respectively. Among the three samples groups, the (Thy+ Ch) samples displayed the lowest FFA.

The above results agree to Serdaroglu and Felekoglu (2005), Ozogul et al. (2010), Ucak et al. (2011) and Emir Çoban et al. (2014). Arise in FFA values during storage is a result of enzymatic hydrolysis as well as lipid oxidation (Ashton, 2002 Barthelet et al., 2008 and Ucak et al., 2011).

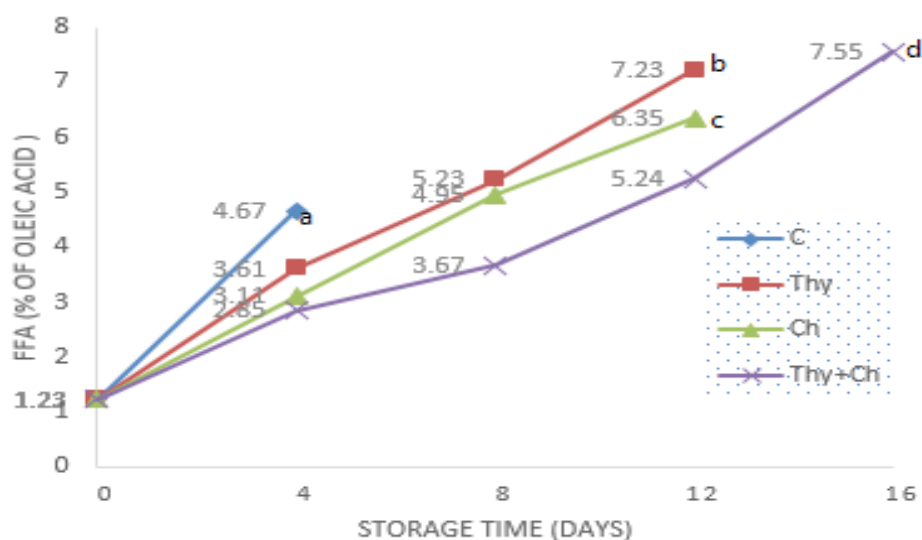
#### Microbiological analyses of fish samples

The Psychrotrophic and *Enterobacteriaceae* counts of the bass treated samples stored in cool

are showed in Fig. 5 and 6, respectively.

The Psychrotrophic and *Enterobacteriaceae* counts at the start of storage were found to be 2.21  $\log_{10}$  cfu/ g and 1, 59  $\log_{10}$  cfu/ g, respectively. Significant ( $P < 0.05$ ) rises in all samples throughout storage were observed (Fig. 5 and 6).

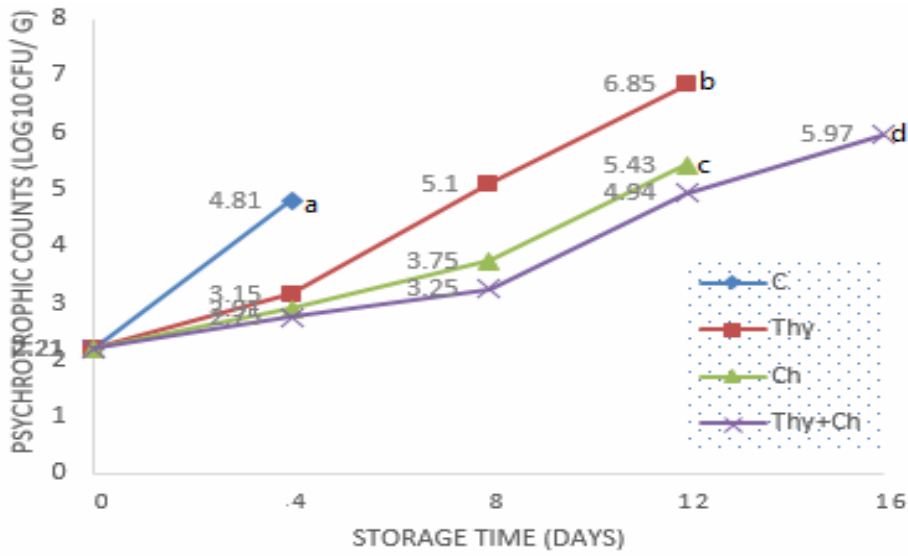
After four days of storage, the Psychrotrophic and *Enterobacteriaceae* counts of the control sample were 4.81 and 3.40  $\log_{10}$  cfu/ g, respectively. Mean while, the other samples solutions have clear bacteriostatic effects and therefore the counts repeated after 8 and 16 days. By day 8, the Psychrotrophic and *Enterobacteriaceae* counts of the samples coated with Thy, Ch and (Thy+ Ch) were 5.10, 3.75 and 3.25  $\log_{10}$  cfu/ g, 3.80, 2.66 and 1.95  $\log_{10}$  cfu/ g, respectively, which still fall within the acceptable levels 6  $\log_{10}$  cfu/g (ICMSF, 2002). Thy + Ch mix exhibited the lowest psychrotrophic and *Enterobacteriaceae* counts. These results may be owing to the ability of chitosan to damage the barrier characteristic of the outside membrane of gram- negative bacteria (Sudarshan et al., 1992)



C: control without any addition. Thy: coated with thyme oil solution. Ch: coated with chitosan solution. Thy+ Ch: coated with mix of thyme oil and chitosan.

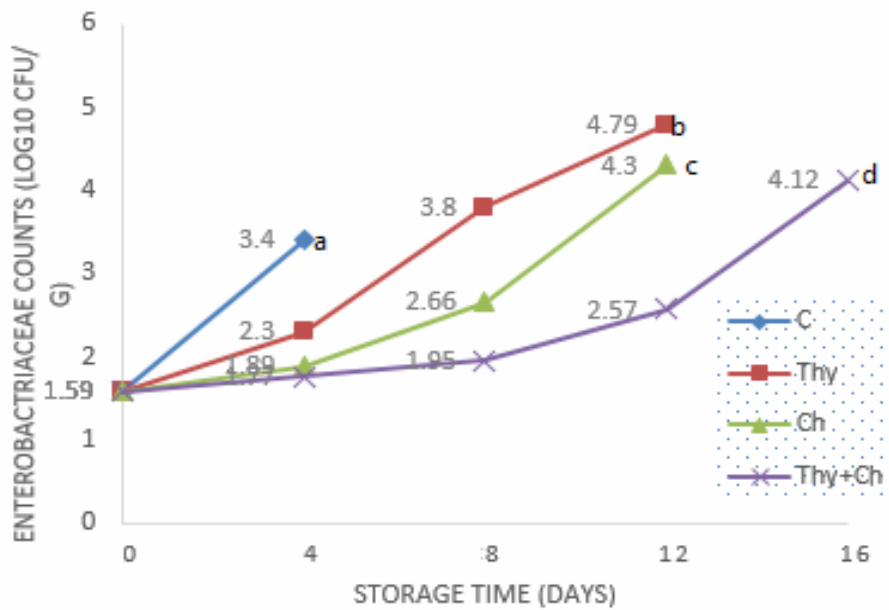
**Fig. 4.** Changes in FFA (% of oleic acid) of coated bass fish fillets with thyme oil solution, chitosan solution and their mixes during refrigerated storage at 4 °C for 16 days. Letters show significant differences between the samples at ( $p < 0.05$ ).





C: control without any addition. Thy: coated with thyme oil solution. Ch: coated with chitosan solution. Thy+ Ch: coated with mix of thyme oil and chitosan.

Fig. 5. Changes in Psychrotrophic counts (log<sub>10</sub> cfu/g) of coated bass fish fillets with thyme oil solution, chitosan solution and their mixes during refrigerated storage at 4 °C for 16 days. Letters show significant differences between the samples at (p<0.05)



C: control without any addition. Thy: coated with thyme oil solution. Ch: coated with chitosan solution. Thy+ Ch: coated with mix of thyme oil and chitosan.

Fig. 6. Changes in Enterobacteriaceae counts (log<sub>10</sub> cfu/g) of coated bass fish fillets with thyme oil solution, chitosan solution and their mixes during refrigerated storage at 4 °C for 16 days. Letters show significant differences between the samples at (p<0.05).

Thus, it could be concluded that the effect of the (Thy+ Ch) coating was able to inhibit the growth of bacteria up to 16 days of cold storage. Consistent with Tsai et al., (2002) who stated that pre-treatment fish (*Oncorhynchus nerka*) for 3hr by 1% chitosan solution delayed the rise within the counts for mesophiles or coliforms in addition to psychrotrophs, *Aeromonas* species or *vibrio* species. Lopez-Caballero et al., (2005) found that blend of chitosan in acetic acid and gelatin exerted an inhibitory control of gram-negative bacteria of fish patties. Moreover, the effect of the Thyme is due to its composition and the presence of antioxidants such as phenolic compounds, (Donmez and Tepe, 2003 and Karabagias et al., 2011). Rasooli et al. (2006) showed that both thyme and rosemary had a bactericidal effect on *listeria monocytogens*.

Varied factors have an effect on the antimicrobial action of chitosan. The mechanism seems to be linked to relations amongst positively charged chitosan particles and negatively charged microbiological cell membranes which effect of the leakage of proteinaceous besides additional intracellular constituents (Papineau et al., 1991, Sudarshan et al., 1992, Chen et al., 1998, Jeon et al., 2002, Kyraana & Lougovois, 2002 and Sallam, 2007).

#### *Colour and odour evaluation fish samples*

The changes detected in the colour and odour, of sea bass samples are presented in Fig. 7 and 8. The colour and odour evaluation at the start of storage were determined to be 9 and 8.7, respectively. Significant ( $P < 0.05$ ) reduction have been observed in all samples throughout storage at 4°C for 16 days (Fig. 7 and 8).

The sample coated with Thy+ Ch was the best after storage bass fish for 16 days, while control sample had storage period for only four days, and could not be accepted by the panelists after more time.

Moreover, samples treated with Thy and Ch had a shelf-life for 12 days.

Similar results for colour and odour evaluation were obtained by Kyraana and Lougovois (2002), Alasalvar et al. (2001) and Ahmad et al. (2012) reported that sea bass fish cuts covered in gelatin film containing lemongrass presented less variations in colour, maybe owing to the antimicrobial as well as antioxidant characteristic of lemongrass.

Other workers showed that, treatment with Ch significantly lowering the microbial counts and modify the quality during storage period (Augustini and Sedjati 2007).

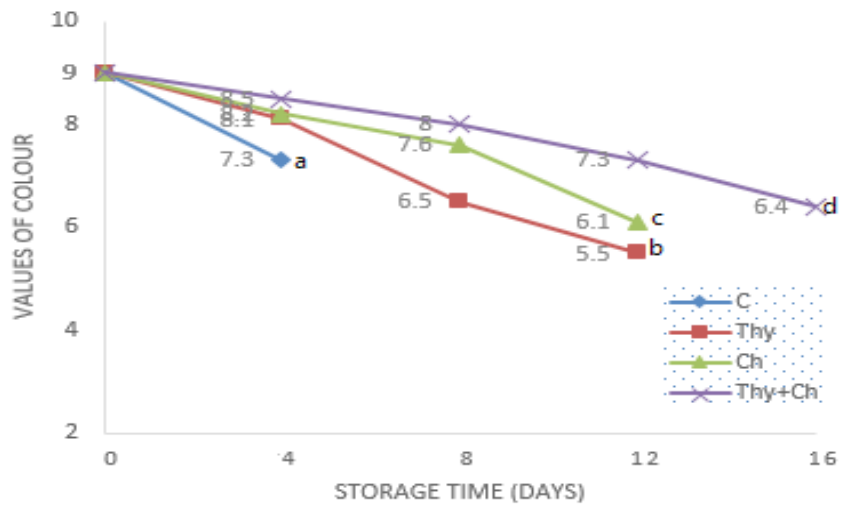
Comparable results have been stated in fish products preserved by natural sources like essential oils be able to usage as a safe technique for storage (Corbo et al., 2009 and Mahmoudzadeh et al., 2010).

#### **Conclusion**

Food stuffs need safety against bacteriological spoilage through storage. Customers request safe natural foods. Natural additives like essential oils be able to usage as a safe technique for storage fish and edible coating samples are confirmed to prolong storage of seafood through usage the natural sources.

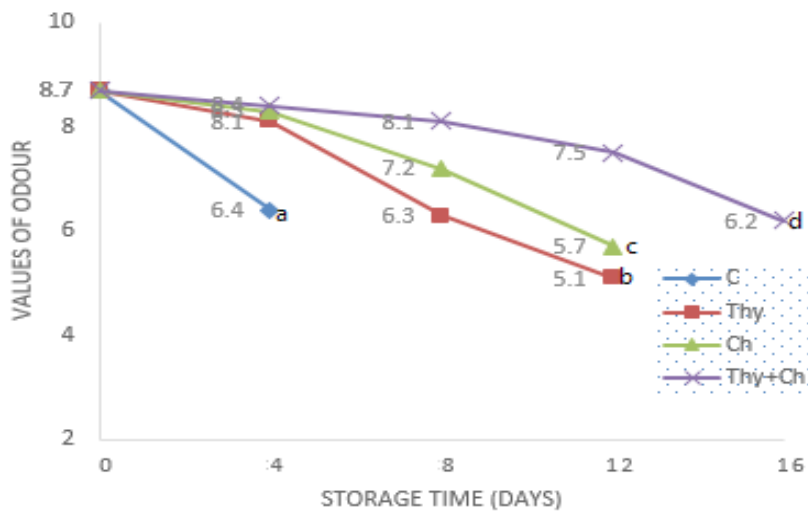
The possible properties of these samples are delay fat deterioration, inhibited bacteriological progress as well as improved sensorial properties. Because of their antimicrobial in addition as antioxidant characteristic, essential oils are favorable their use in place of synthetic compounds. Moreover, the current work showed that, the treatment with thyme oil (Thy), chitosan (Ch) fish fillets could successfully retard bacteriological development, delay chemical damage, keep or increase sensory characteristics, and prolong the storage time. The addition (Thy+ Ch) showed positive effect on the product storage period.

However, addition the combination (Thy+ Ch) be able to delay unwanted chemical changes and retard fat oxidation enhance increase sensory attributes as well as prolong the storage time of the product through cooled storage at 4°C for 16 days.



C: control without any addition. Thy: coated with thyme oil solution. Ch: coated with chitosan solution. Thy+ Ch: coated with mix of thyme oil and chitosan.

Fig. 7. Changes in values of colour of coated bass fish fillets with thyme oil solution, chitosan solution and their mixes during refrigerated storage at 4 °C for 16 days. Letters show significant differences between the samples at (p<0.05).



C: control without any addition. Thy: coated with thyme oil solution. Ch: coated with chitosan solution. Thy+ Ch: coated with mix of thyme oil and chitosan.

Fig. 8. Changes in values of odour of coated bass fish fillets with thyme oil solution, chitosan solution and their mixes during refrigerated storage at 4 °C for 16 days. Letters show significant differences between the samples at (p<0.05).

## References

- Ahmad, S., Benjakul, P. and Sumpavapol, N.P. (2012). Quality changes of sea bass slices wrapped with gelatin film incorporated with lemongrass essential oil, *Int. J. Food Microbiol.* **155**:171–178.
- Alasalvar, C., Taylor, K.D.A., Oksuz, A., Garhtwaite, T., Alexis, M. and Grigorakis, K. (2001) Freshness assessment of cultured sea bream (*Sparus aurata*) by chemical, physical and sensory methods. *Food Chem.* **72**, 33–40.
- Alasalvar, C., Taylor, K.D.A., Zubcov, E., Shahidi, F. and Alexis, M. (2002) Differentiation of cultured and wild sea bass (*Dicentrarchus labrax*): total lipid content, fatty acid and trace mineral composition. *Food Chemistry*, **79**, 145–150.
- AOAC. (2002) *Official Methods of Analysis of AOAC International* (17<sup>th</sup> ed). Washington, DC: Association of Official Analytical Chemists.
- Appendini, P. and Hotchkiss, J. H. (2002) Review of antimicrobial food packaging. *Inno. Food Sci. Emerg. Technol.* **3**, 113–126.
- Ashton, I.P. (2002) Understanding lipid oxidation in fish. In Bremner H.A. (Ed.): *Safety and Quality Issues in Fish Processing*. Washington, DC, CRC Press.
- Augustini, T.W. and Sedjati, S. (2007) The effect of chitosan concentration and storage time on the quality of salted-dried anchovy (*Stolephorus heterolobus*). *J. Coastal Dev.* **10**, 63–71.
- Banerjee, S. (2006) Inhibition of mackerel (*Scomber scombrus*) muscle lipoxigenase by green tea polyphenols. *Food Research International*, **39**: 486–491.
- Barthet, V. J., Gordon, V. and Dany, J. (2008) Evaluation of a colorimetric for method measuring the content of FFA in marine and vegetable oils. *Food Chem.*, **111**, 1064–1068.
- Benjakul, S., Visessanguan, W., Phongkanpai, V. and Tanaka, M. (2005) Antioxidative activity of caramelisation products and their preventive effect on lipid oxidation in fish mince. *Food Chemistry*, **90**, 231–239.
- Campos, I., Matos, E. and Valente, I. (2019) Ability of European seabass (*Dicentrarchus labrax*) to digest rendered animal fats from fish, poultry and mammals. *Aquaculture Nutrition*, **25**, 729–736.
- Chen, C.S., Liau, W.Y. and Tsai, G.J. (1998) Antibacterial effects of Nsulfo benzoyl chitosan and application to oyster preservation. *J. Food Prot.*, **61**, 1124–1128.
- Egypt. *J. Food.* **47**, No.1 (2019)
- Chouliara, E., Karatapanis, A., Savvaidis, I.N. and Kontominas, M.G. (2007) Combined effect of oregano essential oil and modified atmosphere packaging on shelf-life extension of fresh chicken breast meat, stored at 4 C. *Food Microbiol.* **24**, 607–617.
- Connell, J.J. (1990) Methods of assessing and selecting for quality. In *Control of Fish Quality*, 3rd ed., Fishing News Books, Oxford, U.K.
- Connell, J.J. (1995) *Control of Fish Quality*, 4th ed. Fishing News Books Limited, London.
- Corbo, M.R., Di Giulio, S., Conte, A., Speranza, B., Sinigaglia, M. and Del Nobile, M.A. (2009) Thymol and modified atmosphere packaging to control microbiological spoilage in packed fresh cod hamburgers. *International Journal of Food Science and Technology*, **44**, 1553–1560.
- Donmez, E. and Tepe, B. (2003) Antimicrobial and antioxidant activity of the essential oil and methanol extracts of Thymus. *J. Agric. Food Chem.* **51**, 63–67.
- Duan, J., Cherian, G. and Zhao, Y. (2010) Quality enhancement in fresh and frozen lingcod (*Ophiodon elongatus*) fillets by employment of fish oil incorporated chitosan coatings. *Food Chem.* **119**, 524–532.
- Duncan, D.B. (1955) Multiple ranges and multiple f test. *Biometrics*, **11**, 1–42.
- Egan, H., Kirk, R.S and Sawyer, R. (1981) *Person's Chemical Analysis of Foods*. 8th ed.-Longman Scientific and Technical, Longman House. Burnt Mill, Harlow, Essex, CM20.2TE, England.
- Emir Çoban, O., Patir, B. and Yilmaz, O. (2014) Protective effect of essential oils on the shelf life of smoked and vacuum packed rainbow trout fillets. *Journal of Food Science and Technology*, **51**, 2741–2747.
- Erkan, N., Ulusoy, Ş. and Tosun, Y. (2011) Effect of combined application of plant extract and vacuum packaged treatment on the quality of hot smoked rainbow trout. *Journal für Verbraucherschutz und Lebensmittelsicherheit*, **6**, 419–426.
- Erkan, N., Dogruyol, H., Gunlu, A. and Genç, I. (2015) Use of natural preservatives in seafood: plant extracts, edible film and coating. *J. Food Health Sci.* **1** (1), 33–49.
- Etemadian, Y., Shabanpour, B., Mahoonak, A.S. and Shabani, A., (2012) Combination effect of phosphate and vacuum packaging on quality parameters of Rutilus frisii kutum fillets in ice. *Food Research International*, 459–16.

- Falguera, V., Quintero, J.P., Jimenez, A., Munoz, J.A. and Ibarz, A. (2011) Edible films and coatings: Structures, active functions and trends in their use. *Trends in Food Science & Technology*, **22**, 292-303.
- Fan, W., Chi, Y. and Zhang, S. (2008) The use of a tea polyphenol dip to extend the shelf life of silver carp (*Hypophthalmichthys molitrix*) during storage in ice. *Food Chemistry*, **108**, 148-153.
- Fan, W., Sun, J., Chen, Y., Qiu, J., Zhang, Y. and Chi, Y. (2009) Effects of chitosan coating on quality and shelf life of silver carp during frozen storage. *Food Chem.* **115**, 66-70.
- FAO (2018) Global aquaculture production. FAO, Rome. 29 p.
- Fasseas, M.K., Mountzouris, K.C., Tarantilis, P.A. Polissiou, M. and Zervas, G. (2008) Antioxidant activity in meat treated with oregano and sage essential oils. *Food Chem.* **106**, 1188-1194.
- Feng, S.A., Lin, B.F. and Liang, X.Q. (2004) Research development of chitosan film in food preservation. *Polym Bull.* **6**, 68-72.
- Folch, J., Lees, M., and Sloane Stanley, G.H. (1957) A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol Chem.* **226**, 497-509.
- Fuentes, A., Fernandez-Segovia, I., Serra, J. and Abarat, J. M. (2010) Comparison of wild and cultured sea bass (*Dicentrarchus labrax*) quality. *Food Chemistry* **119**, pp. 1514-1518.
- Gammone, M., Riccioni, G., Parrinello, G. and Dorazio, N. (2019). Omega-3 polyunsaturated fatty acids: benefits and endpoints. *Nutrients*, **11**, 46.1-16.
- Garci, M., Diaz, R., Puerta, F., Beldarrain, T., Gonzalez, J. and Gonzalez, I. (2010). Influence of chitosan addition on quality properties of vacuum-packaged pork sausages. *Ciênc. Tecnol. Aliment., Campinas*, **30**, 560-564.
- Gomez-Guillen, M.C. and Montero, M.P. (2007). Polyphenol uses in seafood conservation. *American Journal of Food Technology*, **2** (7), 593-601.
- Gonzalez-Fandos, E., Villarino-Rodriguez, A., Garcia Linares, M. C., Garcia-Arias, M.T. and Garcia-Fernandez, M.C. (2005) Microbiological safety and sensory characteristics of salmon slices processed by the sous vide method. *Food Control*, **16**, 77-85.
- Govaris, A., Solomakos, N., Pexara, A. and Chatzopoulou, P.S. (2010) The antimicrobial effect of oregano essential oil, nisin and their combination against *Salmonella enteritidis* in minced sheep meat during refrigerated storage. *Int. J. Food Microbiol.* **137**, 175-180.
- Grigorakis, K., Nikolopoulou, D., Alexis, M. and Gialamas, I. (2005) Sensory, microbiological, and chemical spoilage of cultured common sea bass (*Dicentrarchus labrax*) stored in ice: a seasonal differentiation. *European Food Research and Technology*, **219**, pp. 584-587.
- Gunlu, A. and Koyun, E. (2013) Effects of vacuum packaging and wrapping with chitosan based edible film on the extension of the shelf life of sea bass (*Dicentrarchus labrax*) fillets in cold storage (4°C). *Food Bioprocess Technology*, **6**, 1713-1719.
- Gutierrez, J., Barry-Ryan, C., and Bourke, P. (2008). The antimicrobial efficacy of plant essential oil combinations and interactions with food ingredients. *Int. J. Food Microbiol.* **124**, 91-97.
- Gutierrez, J., Barry-Ryan, C., and Bourke, P. (2009) Antimicrobial activity of plant essential oils using food model media: efficacy synergistic potential and interaction with food components. *Food Microbiol.* **26**, 142-150.
- Hanusova, K., Dobias, J. and laudisova, K. (2009) Effect of packaging films releasing antimicrobial agents on stability of food products. *Czech J. Food Sci.* **27**, 347-349.
- Hematyar, N., Rustad, T., Sampels, S. and Dalsgaard, T. (2019). Relationship between lipid and protein oxidation in fish. *Aquaculture Research*, **50**, 1393-1403.
- Holley, R.A. and Patel, D. (2005). Improvement in shelf-life and safety of perishable foods by plant essential oils and smoke antimicrobials. *Food Microbiol.* **22**, 273-292.
- Houessou, M., Yelouassi, C., Zanmenou, W., Mossi, I., Suanon, F. and Yovo, P. (2019). Nutritional composition of fatty acids and amino acids of the Fermented (*scomberomorus tritor*) in benin. *Science Journal of Chemistry*, **7**, No. 1, pp. 19-25.
- Huss, H. H. (1988) Fresh fish quality and quality changes. FAO Fisheries Series, No. 29, FAO, Rome.
- Huss, H.H. (1995) Quality and quality changes in fresh fish FAO Fisheries Technical Paper, No. 348. Rome, FAO.
- ICMSF. (2002) International commission on microbiological specifications for foods Microorganisms in foods 7<sup>th</sup>. Microbiological testing in food safety management London: Kluwer Academic/Plenum Publishers. Springer.
- Jeon, Y.J., Kamil, J.Y. and Shahidi, F. (2002) Chitosan as an edible invisible film for quality preservation of herring and Atlantic cod. *Journal of Agricultural and Food Chemistry*, **50**, 5167-5178.



- Jhaveri, S.N., Karakoltsidis, P.A., Montecalvo, J. and Constantinides, S.M. (1984) Amounts and types of amino acids in fish. *J. Food Sci.*, **49**, 110–113.
- Karabagias, I., Badeka, A., and Kontominas, M. G. (2011). Shelf life extension of lamb meat using thyme or oregano essential oils and modified atmosphere packaging. *Meat Sci.*, **88**, 109–116.
- Kaya, Y., Turan, H., Erkoyuncu, I. and Sonmez, G. (2006). The storage in chilled conditions of hot smoked bonito. *Journal of Fisheries and Aquatic Science Ege University*, **23**, 457–460.
- Kazeml, S.M. and Rezael, M. (2015) Antimicrobial effectiveness of gelatin-alginate film containing oregano essential oil for fish preservation. *Journal of Food Safety*, **10**, 1-9.
- Kenar, M., Ozogul, F. and Kuley, E. (2010). Effects of rosemary and sage tea extracts on the sensory, chemical and microbiological changes of vacuum-packed and refrigerated sardine (*Sardinapilchardus*) fillets. *International Journal of Food Science and Technology*, **45**, 2366–2372.
- Khodanazary, A., Salati, A., Bohlouli, S., Mohammadi, M. and Sadeghi, E. (2019). Quality enhancement in refrigerated tiger tooth croaker (*Otolithes ruber*) fillets using chitosan coating containing green tea extract. *Iranian Journal of Fisheries Sciences*, **18** (2) 224-241.
- Kilinc, B., Cakli, S., Dincer, T. and Cadun, A. (2007). Effects of phosphates treatment on the quality of frozen-thawed fish species. *Journal of Muscle Foods*, **20** (4), 377–391.
- Kilinceker, O., Dogan, I.S. and Kucukoner, E. (2009) Effect of edible coatings on the quality of frozen fish fillets. *Food Sci. Technol.* **42**, 868–873.
- Kykkidou S., Giatrakou V., Papavergou A., Kontominas M.G. and, Savvaidis I.N. (2009) Effect of thyme essential oil and packaging treatments on fresh Mediterranean swordfish fillets during storage at 4°C. *Food Chemistry*, **115**, 169–175.
- Kyrana, V.R. and Lougovois, V.P. (2002). Sensory, chemical, microbiological assessment of farm-raised European sea bass (*Dicentrarchus labrax*) stored in melting ice. *Int J Food Sci Technol.* **37**, 319-328.
- Lee, H.J., Park, H.J., Jeong, J.W., Kim, D. and Chinnan, M.S. (2007) Effect of electrolyzed water treatments on the quality of hand- and machine-peeled yams (*Dioscorea spp.*) during cold storage. *Food Sci. Technol.* **40**, 646–654.
- Lopez-Caballero, M.E., Gomez-Guillen, M.C, Perez Mateos, M. and Montero, P.A. (2005) Chitosan-gelatin blend as a coating for fish patties. *Food Hydrocol.* **19**:303–311.
- Mahmoudzadeh, M., Motallebi, A., Hosseini, H., Khaksar, R., Ahmadi, H., Jenab, E., Shahraz, F. and Kamran, M. (2010). Quality changes of fish burgers prepared from deep flounder (*Pseudorhombus elevates Ogilby*, 1912) with and without coating during frozen storage (–18 °C). *International Journal of Food Science and Technology*, **45**, 374–379.
- Mangena, T., and Muyima, N. (1999) Comparative evaluation of the antimicrobial activities of essential oils of *Artemisia afra* Pteroniaincana and *Rosemarinus officinalis* on selected bacteria and yeast strains. *Lett. Appl. Microbiol.* **28**, 291-296.
- Marino, M., Bersani, C., and Comi, G. (2001). Impedance measurement to study antimicrobial activity of essential oils from *Lamiaceae* and *Compositae*. *Int. J. Food Microbiol.* **67**, 187-195.
- Masniyom, P., Soottawat, B. and Visessanguan, W. (2005) Combination effect of phosphate and modified atmosphere on quality and shelf-life extension of refrigerated seabass slices. *Journal of Food Science and Technology*, **38**, 745–756.
- Moore, S. (1958) Automatic recording apparatuses use in the chromatography of amino acid. **39**, 119 – 121.
- Mossel, D. A., Eelderink, L., Koopmans, M., and Rossem, F. V. (1979). Influence of carbon source, bile salts, and incubation temperature on recovery of *Enterobacteriaceae* from foods using MacConkey-type agars. *Journal of Food Protection*, **42**, 470–475.
- Ocano-Higuera, V.M., Maeda-Martinez, A.N., Marquez-Rios, E., Canizales-Rodríguez, D.F., Castillo-Yanez, F.J. and Ruiz-Bustos, E., M. (2011). Freshness assessment of ray fish stored in ice by biochemical, chemical and physical methods. *Food Chemistry*, **125**, 49–54.
- Ojagh, S.M., Rezaei, M., Razavi, S.H. and Hosseini, M.H. (2010) Effect of chitosan coatings enriched with cinnamon oil on the quality of refrigerated rainbow trout. *Food Chemistry*, **120**, 193-198.
- Ordonez J.A., Lopez-Galvez D.E., Fernandez M. and Hierro, E. L. (2000) Microbial and physicochemical modifications of hake (*Merluccius merluccius*) steaks stored under carbon dioxide enriched atmospheres. *Journal of the Science of Food and Agriculture*, **80**, 1831–1840.
- Ozden, O. And Erkan, N. (2008) Comparison of biochemical Composition of three aquacultured fishes (*Dicentrarchus labrax*, *Sparusaurata*, *Dentexdentex*). *International Journal of Food Sciences and Nutrition*, **59**, pp. 545–557.
- Egypt. J. Food.* **47**, No.1 (2019)

- Ozogul, F., Gokbulut, C., Ozyurt, G., Ozogul, Y and Dural, M. (2005) Quality assessment of gutted wild sea bass (*Dicentrarchus labrax*) stored in ice, cling film and aluminum foil. *European Food Research and Technology*, **220**, 292–298.
- Ozogul, Y., Ozogul, F., Kuley, E., Ozkutuk, S., Gokbulut, C. and Kose, S. (2006) Biochemical, sensory and microbiological attributes of wild turbot (*Scophthalmus maximus*), from the Black Sea, during chilled storage. *Food Chemistry*, **99**, 752–758.
- Ozogul, Y., Ayas, D., Yazgan, H., Ozogul, F., Boga, E.K. and Ozyurt, G. (2010) The capability of rosemary extract in preventing oxidation of fish lipid. *Int.J. Food Sci. and Tech.*, **45**, 1717–1723
- Ozyurt, G. and Polat, A. (2006) Amino acid and fatty acid composition of wild sea bass (*Dicentrarchus labrax*) a seasonal differentiation. *European Food Res. and Tech.*, **222**, pp. 316–320.
- Pandharipande, S., Jana, R. and Ramteke, A. (2018) Synthesis and characterization of chitosan from fish scales. *International Journal of Science, Engineering and Technology Research (IJSETR)* Volume 7, Issue 4, 287-291.
- Papineau, A.M., Hoover, D.G., Knorr, D., and Farkas, D.F. (1991) Antimicrobial effect of water-soluble chitosan with high hydrostatic pressure. *Food Biotechnol.* **5**, 45-57.
- Parvaneh, V. (2007) Quality control and the chemical analysis of food. Tehr. University of Tehr. Press. Chap. 4.
- Patsias, A., Chouliara, I., Badeka, A., Savvaidis, I. N. and Kontominas, M.G. (2006) Shelf-life of a chilled precooked chicken product stored in air and under modified atmospheres: microbiological, chemical, sensory attributes. *Food Microbiology*, **23**, 423–429.
- Pezeshk, S., Ojagh, S.M. and Alishahi, A. (2015) Effect of plant antioxidant and antimicrobial compounds on the shelf-life of seafood – a review. *Czech J. Food Sci.*, **33**, 195–203.
- Poli, B.M., Parisi, G. and Zampacavallo, G. (2001). Quality outline of European sea bass (*Dicentrarchus labrax*) reared in Italy: shelf life, edible yield, nutritional and dietetic traits. *Aquaculture*, **202**, 303-305.
- Prato, E., Biandolino, F., Parlapiano, I., Giandomenico, S., Denti, G., Calo, M., Spada, L. and Di-Leo, A. (2019) Proximate, fatty acids and metals in edible marine bivalves from Italian market: Beneficial and risk for consumers health. *Science of The Total Environment*, **648**, 153–163.
- Radwan, S.S. (1978) Coupling of two-dimensional quantitative analysis of lipids classes and their constituent fatty acids. *J. Chromatog. Sci.* **16**. 538.
- Rasooli, I., Rezaei, M.B. and Allameh, A. (2006) Ultrastructural studies on antimicrobial efficacy of thyme essential oils on *Listeria monocytogenes*. *Int. J. Infect. Diseases*, **10**, 236-241.
- Roomiani, L., Ghaeni, M., Moarref, M., Fallahi, R. and Lakzaie, F. (2019) The effects of (*Rosmarinus officinalis*) essential oil on the quality changes and fatty acids of (*Ctenopharyngodon idella*.) *Iranian Journal of Fisheries Sciences*, **18** (1) 95-109.
- Sallam, K.I. (2007) Antimicrobial and antioxidant effects of sodium acetate, sodium lactate, and sodium citrate in refrigerated sliced salmon. *Food Control*. **18**, 566–575.
- Serdaroglu, M. and Felekolu, E. (2005). Effect of using rosemary extract and onion juice on oxidative stability of sardine (*Sardina pilchardus*) mince. *Journal of Food Quality*, 28109 – 120.
- Shi, C., Cui, J., Yin, X., Luo, Y. and Zhou, Z. (2014). Grape seed and clove bud extracts as natural antioxidants in silver carp (*Hypophthalmichthys molitrix*) fillets during chilled storage: Effect on lipid and protein oxidation. *Food Control*, **40**, 134-139.
- Shyu, Y., Chen, G., Chiang, S. and Sung, W. (2019). Effect of chitosan and fish gelatin coatings on preventing the deterioration and preserving the quality of fresh-cut apples. *Molecules*, **24**, 1-14.
- Skandamis, P., Tsigarida, E., and Nychas, G. J. (2002). The effect of oregano essential oil on survival/death of *Salmonella typhimurium* meat stored at 5 °C under aerobic, VP/MAP conditions. *Food Microbiol.* **19**, 97–103.
- Solomakos, N., Govaris, A., Koidis, P. and Botsoglou, N. (2008). The antimicrobial effect of thyme essential oil, nisin and their combination against *Escherichia coli* O157: H7 in minced beef during refrigerated storage. *Meat Sci.* **80**, 159-166.
- Sousa, A.K., Fogaça, F.H., Araujo, T.D., Castro, K.N., Pereira, A.M., Canuto, K.M., Brito, E.S., Mai, M.G. and Magalhaes, J.A. (2019) Effect of *Alpinia zerumbet* essential oil on the shelf life of tamaqua fillets during short-term refrigerator storage. *Acta Amazonica*, **49**, 152-161.
- Sudarshan, N.R., Hoover, D.G. and Knorr, D. (1992) Antibacterial action of chitosan. *Food Biotechnol.*, **6**, 257-272.
- Taliadourou, D., Papadopoulos, V., Domvridou, E., Savvaidis, I.N. and Kontominas, M.G. (2003) Microbiological, chemical and sensory changes of

- whole and filleted Mediterranean aquacultured sea bass (*Dicentrarchus labrax*) stored in ice. *J. Sci. Food and Agric.*, **83**, 1373–1379.
- Tarladgis, B. G., Watts, B. M., Younathan, M. S. and Dugan, L. J. (1960). A distillation method for the quantitative determination of malonaldehyde in rancid foods. *Journal of the American Oil Chemists' Society*, **37**, 44–48.
- Tinting, L., Jianrong, L., Wenzhong, H. and Xuepeng, L. (2013). Quality enhancement in refrigerated red drum (*Sciaenops ocellatus*) fillets using chitosan coatings containing natural preservatives. *J. Food Chem.*, **138**, 821–826.
- Tsai, W.H., Su, H.C. and Chen, C.L. (2002). Antimicrobial activity of shrimp chitin and chitosan from different treatments and applications of fish preservation, *Fisheries Sci.*, **68**, 170 – 177.
- Ucak, L., Ozogul, Y. and Durmuş, M. (2011) The effects of rosemary extract combination with vacuum packing on the quality changes of Atlantic mackerel fish burgers. *Int. J. Food Sci. and Technol.*, **46**, 1157–1163.
- Vander, S. C., Jacxsens, L., Devlieghere, F. and Debevere, J. (2002) Combined high Oxygen atmosphere with low oxygen atmospheres packaging to improve the keeping quality of strawberries and raspberries. *Postharvest Biology and Technology*, **26**, 49–58.
- Wesselinova, D. (2000). Essential of amino acids in fish muscle. *J. Aquat Food Product. Technol.*, **9** (4), 41–48.

## تأثير استخدام الشيتوزان والزعتر أثناء التخزين المبرد لأسماك القاروص

أيمن محمد أبو اليزيد

قسم علوم الاغذية - كلية الزراعة ساجا باشا - جامعه الاسكندريه

تهدف هذه الدراسة استخدام بعض المواد الحافظة الطبيعية مثل زيت الزعتر والشيتوزان من خلال دراسته الجودة و التخزين لأسماك القاروص في  $4 \pm 1$  درجة مئوية لمدة 11 يوماً. شرائح السمك المغطاه بزيت الزعتر (Thy) أو الشيتوزان (Ch) ومزيج من زيت الزعتر والشيتوزان (Thy + Ch) بينما C (كنترول دون أي إضافة).

وتم تحليل العينات كل أربعة أيام. أظهرت النتائج أن معظم الأحماض الأمينية تشير إلى ان الجلوتاميك و حمض الأسبارتيك كان من اعلي القيم. كما كان هناك محتويات عالية من الأحماض الأمينية التي لا غنى عنها مثل ليسين إلى جانب الليوسين. علاوة على ذلك . أظهرت الأحماض الدهنية أن حمض البالمتيك (23.57%) كان الحمض الدهني الرئيسي المشيع بينما تشتمل الأحماض الدهنية المتعددة غير المشبعة الرئيسية على حمض اللينوليك وحمض الدوكوزاهيكسينويك (DHA) وحمض الإيكوسابنتاينويك (EPA).

أظهرت التحليلات الكيميائية للنتائج في البدايه كانت درجة الحموضة أيضا TVB-N بالإضافة إلى TBA وكذلك FFA كانت 6.48، 8.59 ملغم / 100 جم، 0.11 ملغ مالونالدهيد / كجم و 1.23% (معبراً عنه كنسبة مئوية من حمض الأوليك) . على التوالي. وكانت Psychrotrophic و *Enterobacteriaceae* هي 2.21  $\log_{10}$  cfu / g و 0.59  $\log_{10}$  cfu / g . على التوالي. أثناء التخزين . لوحظت زيادة تدريجية في كل العينات . وتم الحصول على اختلافات بين العينات ( $P < 0.05$ ).

كشفت نتائج التحليل الحسي أن فترة تخزين شرائح السمك كانت أربعة أيام للكنترول . 11 و 12 يوماً للمعاملات بـ (Thy) و (Ch) و (Thy + Ch) على التوالي ( $P < 0.05$ ). أخيراً إضافة (Thy + Ch) يعطي نتيجة إيجابية في فترة تخزين المنتجات السمكية. ومع ذلك فإن إضافة خليط (Thy + Ch) يمكن أن يؤخر التغيرات الكيميائية وأيضاً أكسدة الدهون ويزيد من الصفات الحسية ويطيل تخزين المنتج خلال التخزين المبرد بدرجة حرارة 4 مئوية لمدة 11 يوم.