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.

This study aimed to apply and study the effect of natural preservatives thyme oil and chitosan on the storage of sea bass at 4±1°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, Thioarbituritic 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 log10 cfu/g and 1.59 log10 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°C ±1°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 contented 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 peopole (Alasalvar et al., 2002). Cooling is one of the most well – known preservation technique, which may not sufficient to
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 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 (Mangen 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 Choulia 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-Caballeria 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 ± 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 ± 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 Tylencorphynchus 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± 1°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 °C and 550 °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 (Miny 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 °C, siloxane capillary column HP – 5 (30m x 0.32 mm I.D. × 0.25 μm 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 Ltd) 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 (log10 CFU/g).

_References_

**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 ± 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 ±0.53%, while the protein was 18.85±0.34%, Total lipid was 2.50 ±0.29 % and ash was 1.51±0.61% 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).**

<table>
<thead>
<tr>
<th>Chemical constituents</th>
<th>g/100 g fish</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>77.30 ± 0.53</td>
</tr>
<tr>
<td>Crude protein</td>
<td>18.85 ± 0.34</td>
</tr>
<tr>
<td>Total lipid</td>
<td>2.50 ± 0.29</td>
</tr>
<tr>
<td>Ash</td>
<td>1.51 ± 0.61</td>
</tr>
</tbody>
</table>

Values are mean ± standard error from triplicate determinations.

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

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

Mean values ± 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 is consists of 35.67% saturated (SFA), 32.31% monounsaturated (MUFA) and 32.02% polyunsaturated (PUFA). The main fatty acids in bass fillets were palmitic acid (C16:0), oleic acid (C18:1) and eicosapentaenoic acid (EPA, C20:5 ω3) as well as docosahexaenoic acid (DHA, C22:6 ω3), 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 ω-3 to ω-6 fatty acids was (1.91) which is similar for farmed bass of Greece and Spain (Fuentes et al., 2010). While, the proportion of ω-3 to ω-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 alkaline 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)**

<table>
<thead>
<tr>
<th>Fatty acids</th>
<th>(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C14:0</td>
<td>1.64±0.36</td>
</tr>
<tr>
<td>C16:0</td>
<td>1.53±0.86</td>
</tr>
<tr>
<td>C18:0</td>
<td>23.57±0.34</td>
</tr>
<tr>
<td>C18:1</td>
<td>1.93±0.73</td>
</tr>
<tr>
<td>C18:2</td>
<td>6.15±0.22</td>
</tr>
<tr>
<td>C20:0</td>
<td>0.85±0.98</td>
</tr>
<tr>
<td>∑SFA</td>
<td>35.67±0.77</td>
</tr>
<tr>
<td>C14:1</td>
<td>0.35±0.42</td>
</tr>
<tr>
<td>C16:0</td>
<td>5.34±0.32</td>
</tr>
<tr>
<td>C16:1</td>
<td>1.72±0.73</td>
</tr>
<tr>
<td>C18:0</td>
<td>21.89±0.84</td>
</tr>
<tr>
<td>C20:1</td>
<td>1.59±0.23</td>
</tr>
<tr>
<td>C20:2</td>
<td>1.42±0.64</td>
</tr>
<tr>
<td>∑MUFA</td>
<td>32.31±0.70</td>
</tr>
<tr>
<td>C16:2</td>
<td>9.24±0.54</td>
</tr>
<tr>
<td>C16:3</td>
<td>1.52±0.21</td>
</tr>
<tr>
<td>C20:2</td>
<td>0.82±0.13</td>
</tr>
<tr>
<td>C20:3</td>
<td>ND</td>
</tr>
<tr>
<td>C20:4</td>
<td>0.94±0.68</td>
</tr>
<tr>
<td>C20:5</td>
<td>6.61±0.79</td>
</tr>
<tr>
<td>C22:6</td>
<td>12.89±0.46</td>
</tr>
<tr>
<td>∑PUFA</td>
<td>32.02±0.66</td>
</tr>
<tr>
<td>∑ω-3</td>
<td>21.02</td>
</tr>
<tr>
<td>∑ω-6</td>
<td>11.00</td>
</tr>
<tr>
<td>∑ω-3/ω-6</td>
<td>1.91</td>
</tr>
</tbody>
</table>

Mean values ± Standard Deviation Values
ND = Not determined.

**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 monoethylyamine in addition dimethylanine, 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/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 g respectively. That yet within the acceptable.

The samples treated by (Thy+ Ch) lower of the TVB-N values contrast with the treatment coated by Thy or 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 / 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 byThy 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).


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).

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 Barthet et al., 2008 and Ucak et al., 2011).

Microbiological analyses of fish samples

The Psychrotrophic and Enterobactriaceae counts of the bass treated samples stored in cool are showed in Fig. 5 and 6, respectively.

The Psychrotrophic and Enterobactriaceae counts at the start of storage were found to be 2.21 log10 cfu/ g and 1, 59 log10 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 Enterobactriaceae counts of the control sample were 4.81 and 3.40 log10 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 Enterobactriaceae counts of the samples coated with Thy, Ch and (Thy+ Ch) were 5.10, 3.75 and 3.25 log10 cfu/ g, 3.80, 2.66 and 1.95 log10 cfu/ g, respectively, which still fall within the acceptable levels 6 log10 cfu/g (ICMSF, 2002). Thy + Ch mix exhibited the lowest psychrotrophic and Enterobactriaceae 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)

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).

Fig. 5. Changes in Psychrotrophic counts (log_{10} 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).


Fig. 6. Changes in Enterobactriaceae counts (log_{10} 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 (Oncomorhynchus nerka) for 3hr by 1% chitosan solution delayed the rise within the counts for mesophiles or coliforms in addition to psychrotrophs, Acromonas 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, Kyrana & 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 Kyrana 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.

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).


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


Taliadourou, D., Papadopoulos, V., Domvridou, E., Savvidais, I.N. and Kontominas, M.G. (2003) Microbiological, chemical and sensory changes of...
تأثر استخدام الشيتوزان والزعتر أثناء التخزين المبرد لأسماك القرص

أيمن محمد أبوالزيد
قسم علوم الأغذية - كلية الزراعه سباها - جامعة الأسكندرية

وهدف هذه الدراسة استخدام بعض المواد الخاصة الطبيعية مثل زيت الزعتر والشيتوزان من خلال دراسة التخزين و الحفاظ لأسماك القرص في درجة مئوية لمدة 11 يوما. شرائح السمك بدرجة مئوية (Thy) (C) (كنثرون دون أي إضافات) وشرائح السمك مع زيت الزعتر والشيتوزان (Ch) (C) (ذين كنت محتويات إلى C، أثناء التخزين). أظهرت النتائج أن معظم الأحماض الأمينية تشير إلى الأحماض إنزيمات، حيث كانت مستقلة عن النبيسيتين. كما أن هناك اختلافات عالية من الأحماض الأمينية التي 4% من خلال النبيسيتين. الأحماض إنزيمات الذين تشير إلى النبيسيتين غدرية الشريحة الرئيسية على حمض الليبيول. 

الأحماض إنزيمات (Omega-3) ذات الصلة مع المحاصيل، حيث كانت مستقلة عن النبيسيتين. كما أن هناك اختلافات عالية من الأحماض الأمينية التي 4% من خلال النبيسيتين. الأحماض إنزيمات الذين تشير إلى النبيسيتين غدرية الشريحة الرئيسية على حمض الليبيول. 

النتائج على اختلافات بين العينات (P<0.05).

النتائج على اختلافات بين العينات (P<0.05).

かった في إنزيمات حيث(Note: It's unclear if there's a typo in "(P)<0.05" as the note suggests a correction to "(P)<0.05".)


