Utilization of Some Essential Oils to Extend The Shelf-Life of Coated Semi Fried Nile Perch Fish Fillets during Cold Storage

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ABSTRACT

This research was performed to evaluate the antioxidant and antimicrobial effects of marjoram (Marjorana hortensis), mint (Mentha piperita) and thyme (Thymus capitatus) essential oils as natural preservatives to extend the shelf-life of coated semi fried Nile perch (Lates niloticus) fish fillets. Skinless Nile perch fish fillets were treated with two ratios; 0.5 (T1) and 1.0 (T2) ml of marjoram essential oil per 100g fish fillets, 1.5 (T3) and 2.0 (T4) ml of mint essential oil per 100g fish fillets and with 0.5 (T5) and 1.0 (T6) ml of thyme essential oil per 100g fish fillets. The treated fish fillets with tested essential oils and untreated one (control sample) were coated with dough mixture (wheat flour, cold water, whole egg, sodium chloride, cumin powder and xanthan gum) and fried at 170±5ºC for 1 mint. The coated semi fried fillets were packed in polyethylene bags and after that stored at 4±1 ºC for 16 days. The physico-chemical quality criteria; total volatile basic-nitrogen (TVB-N) content, trimethylamine-nitrogen (TMA-N) content, thiobarbituric acid (TBA) value, acid value and the pH value, microbiological quality indices; the total viable bacteria (TVB), psychrotrophic bacteria, pseudomonas spp. bacteria and yeasts and molds counts, and sensory analysis were used to evaluate the preservative effect of tested essential oils during storage at 4±1 ºC for 16 days. The present results showed that there was a significant (P≤0.05) increase in TVB-N, TMA-N, TBA and acid values in different ratios depending on the ratio and the kind of tested essential oil itself. The lowest significant incremental rate was recorded in samples (T2). All treatments, except (T3), gave significantly (P≤0.05) reduction in the TVB-N values and psychrotrophic, and pseudomonas spp. bacteria counts immediately after treatment as compared with control treatment. The marjoram, mint and thyme essential oils in both ratios were able significantly to reduce the yeast and mold counts immediately at zero time .The increase in all tested microbiological quality criteria continued during the cold storage in all treatments which was lower than control sample. The obtained results also showed that there was a significant (P≤0.05) enhancement in sensory quality attributes of fish fillets’ samples treated with marjoram, mint and thyme essential oils at both tested ratios. In general, the tested essential oils’ treatments caused a significant (P≤0.05) extending of the shelf-life, as well as enhancement the physico-chemical, microbiological and sensory quality attributes for coated semi fried Nile perch fish fillets during cold storage. In addition, fish samples treated with 1.0 ml marjoram essential oil (T2) followed by samples treated with 1.0 ml thyme essential oil (T4) per 100 g fish fillets attained the highest scores for sensory quality attributes and exhibited the better acceptability than the control and the other treated fish samples. Therefore, the present findings recommended that the essential oils of marjoram, mint and thyme should be utilized for extending the shelf-life and enhancing quality attributes of coated semi fried fish fillets during cold storage.

Key words: Essential oils, Shelf-life, Semi fried fish, Quality criteria, Cold storage.

Introduction

Fish and fishery products have been recognized as nutrition source due to their high protein content. However, shelf-life of seafood is limited by biochemical and microbial changes, which causes substantial practical problems in distribution (Mahmoud et al., 2004 and Masniyom, 2011). Microorganisms such as bacteria, mold and yeast are known to be responsible for putrefaction and development of poor marketing appearance and toxic substances in fish that may be passed to consumers (Omojowo and Sogbesan, 2003). The another important issue is oxidative rancidity that lead to the qualitative deterioration (Karpinska et al., 2001). Spices and their essential oils are the most efficient natural antioxidant and antimicrobial agents have long been used to preserve food (Burt, 2004).

Essential oils are aromatic and volatile liquids extracted from plant. The chemicals in essential oils are secondary metabolites, which play an important role in plant defense as they often possess antimicrobial properties. The interest in essential oils and their application in food preservation have been amplified in recent
years by an increasingly negative consumer perception of synthetic preservatives. Furthermore, food-borne diseases are a growing public health problem worldwide, calling for more effective preservation strategies (Hyldgaard et al., 2012).

The fact that many essential oils possess antimicrobial activity has been proved by plenty of investigations (Skrinjar and Nemet, 2009). Many studies were carried out to prove the effect of combination of refrigeration and essential oils on extending the shelf-life of fish and fish products (Harpaz et al., 2003; Goulas and Kontominas, 2007; Selmi and Sadok, 2008; Can, 2012 and Zakipour and Divband, 2012). Essential oils of marjoram, mint and thyme are used as an antimicrobial and antioxidant in many food products. Essential oils of thyme and oregano herbs at the ratio of 0.05% (v/v) were used as preservatives for Asian sea bass fish which stored at 0 ºC to 2 ºC. The shelf-life was extended from 25 days to 33 days for non-treated and treated fish; respectively (Harpaz et al., 2003). Another study carried out by Erkan et al. (2011) found that the use of thyme (1%) and laurel essential oil (1%) was extended the shelf-life of bluefish by about 3–4 days. The oils of dill, marjoram and geranium could be used in food manufacturing such as sausage, fish and fishery products to increase the acceptability of these products, in addition to their effect on the shelf-life (Effat-Afifi, 2001). Mint essential oil is a promising additive for those who seek natural antimicrobial systems for food preservation. Its antibacterial action depends mainly on the food pH, composition, storage temperature and the nature of the microorganisms (Tassou et al., 1995).

Nile perch (Lates niloticus) is a species of freshwater fish in the family of Centropomidae of the order Perciformes (Warui, 2007). The Nile perch is the most economically important fish species in Egypt living in tropical and semitropical waters (Mousa, 2001).

Therefore, this study was carried out to investigate the effect of marjoram, mint and thyme essential oils on the physico-chemical, microbial and sensory quality criteria of coated semi fried Nile perch fish fillets during cold storage, at 4±1 ºC for 16 days, as well as to determine the effect of the former tested essential oils treatment on the self-life of coated semi fried fish fillets, under investigation, during cold storage.

Materials and Methods

Materials:

Fresh Nile perch (Lates niloticus) fish was obtained from local market, Cairo city, during May, 2012 season. The fish were approximately 800 – 900g weight each. The fish were put in ice box and transferred to Food Science and Technology Department, Faculty of Agriculture, Cairo University. The edible coating dough ingredients were wheat flour, cold water, whole egg, sodium chloride, cumin powder, which were obtained from local market except xanthan gum (bacterial source) was obtained from Sigma Chemical Co. (St. Louis, Mo.). The essential oils of marjoram, mint and thyme spices were distilled from their spices by steam distillation, obtained from Kato Aromatic Co., Giza, Egypt.

Preparation of Coated Semi Fried Fish Fillets:

Fish samples were washed, gutted, filleted, rewashed and drained. Fillets were divided into seven batches. One batch was dipped into the edible coating dough and recorded as control sample. To evaluate the effects of investigated antimicrobial and antioxidant essential oils, the essential oil was mixed with corn oil and spread on 100g fish fillet surface; 0.5 ml of marjoram essential oil plus 10 ml of corn oil (treatment No. 1); 1.0 ml of marjoram essential oil plus 10 ml of corn oil (treatment No. 2); 1.5 ml of mint essential oil plus 10 ml of corn oil (treatment No. 3); 2.0 ml of mint essential oil plus 10 ml of corn oil (treatment No. 4); 0.5 ml of thyme essential oil plus 10 ml of corn oil (treatment No. 5); 1.0 ml of thyme essential oil plus 10 ml of corn oil (treatment No. 6). After that, all six treated fish fillets’ samples were mixed with coating dough. All coated fish fillets’ samples were left for 3-4 minutes at ambient temperature and then semi fried, using an electrical deep fryer (General electric), in corn oil heated at 170±5 ºC for 1 minute, then drained in basket to remove excess oil. Different coated fish fillet samples were packed in polyethylene bags and stored at 4±1 ºC for 16 days.

Chemical Analysis:

Proximate chemical composition:

Proximate chemical composition (moisture, protein, fat and ash contents) of fresh and coated semi fried fish fillets was determined according to the methods described by A O A C (2000).
Determination of total volatile basis nitrogen (TVB-N) and trimethylamine nitrogen (TMA-N) contents:

The TVB-N and TMA-N contents were determined as described by Malle and Poumeyrol (1989).

Determination of thiobarbituric acid (TBA) value:

Direct determination of the TBA value in trichloroacetic acid extracts of flesh fish was determined according to the method reported by Vyncke (1970).

Determination of acid value:

The acid values of fat extracted from samples were measured by method described by Cox and Pearson (1962) by dissolving the fat in 50 ml of the standard solvent (Mixing 25 ml ether, 25 ml 95% alcohol and 1 ml of 1% phenolphthalein solution and neutralize with 0.1N potassium hydroxide (KOH) in a 250 ml conical flask and then a few drops of phenolphthalein were added. The contents were titrated against 0.1 N KOH solution and shacked constantly until a pink color which persists for fifteen seconds is obtained. Acid value was calculated by using following equation:

\[ \text{Acid value (mg KOH/g)} = \frac{\text{Titrate value} \times \text{Normality of KOH} \times 56.1}{\text{Weight of sample (g)}} \]

Microbial Analysis:

Total viable bacteria (TVB), Psychrotrophic bacteria and Pseudomonas spp. bacteria were enumerated as described by Erkan et al. (2011). Yeasts and molds count was enumerated as described by ICMSF (1986).

Sensory Evaluation:

Panel group of ten members randomly selected from the staff members of the Dept. of Food Sci. and Tech. Fac. of Agric., Cairo Univ. evaluated the coated semi fried Nile perch fish fillets after frying in corn oil at 170°C for 5 minutes according to Larmond (1973). Panelists were asked to score appearance, color, odor, taste, tenderness, Juiciness and overall acceptability according to 9 - point hedonic scale.

Statistical Analysis:

Data were presented as mean and standard deviation (SD) values. Student's t-test was used to compare between chemical composition of raw and coated semi-fried fish. One-way Analysis of Variance (ANOVA) was used to compare between different treatments. Repeated measures ANOVA test was used to study the changes by time within each treatment. Tukey's post-hoc test was used for pair-wise comparison between the mean values when ANOVA test is significant. The significance level was set at P ≤ 0.05. Statistical analysis was performed with IBM® SPSS® Statistics Version 20 for Windows according to the procedure of Riffenburgh (2006).

Results and Discussion

1. Proximate Chemical Composition for Fresh and Coated Semi Fried Nile Perch Fish Fillets:

Table (1) shows the changes in chemical composition of coated semi fried fish fillets as a result of heat treatment.

<table>
<thead>
<tr>
<th>Component %</th>
<th>Chemical Component (% ; M±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fresh fillets</td>
</tr>
<tr>
<td>Protein</td>
<td>91.1±0.5 a</td>
</tr>
<tr>
<td>Fat</td>
<td>6.19±0.3 a</td>
</tr>
<tr>
<td>Ash</td>
<td>1.35±0.03 a</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>1.40±0.04 a</td>
</tr>
</tbody>
</table>

M±SD: Mean of triplicate determinations for gross chemical component; the means, within the same row, having different superscripts are varied significantly (p ≤ 0.05). Moisture content was 80.78±0.3 and 75.37±0.7 for fresh fillets and coated semi fried fillets; respectively.

For fresh fillets the moisture, protein, fat, ash and carbohydrates contents were 80.78, 91.1, 6.19, 1.35 and 1.40%; respectively (on dry basis). From fat result, the Egyptian Nile perch fish is considered as a non-fatty fish.
This proximate chemical composition is nearly accordance with that obtained by Okeyo et al. (2009) for Kenyan Nile perch fish fillets. Meanwhile, the corresponding values in semi fried fillets were 75.37, 64.96, 12.79, 5.89 and 16.36, respectively. The semi fried process increased the percentages of moisture and protein losses by 6.69% and 28.69%, respectively. Similar results were obtained by Yasin and Abou-Taleb (2007). This loss in moisture content of semi fried fillets was due to evaporation of moisture and elution of components into fried oil and loss of protein, it may be attributed to decomposition and conversion of some protein molecules to volatile nitrogen due to further rise of temperature that leads to protein hydrolysis as described by Mostafa et al. (2002).

Otherwise, the fat, ash and total carbohydrates were increased; as given in Table (1). This incremental pattern in the aforementioned parameters might be due to the constituents of coated layer which contained polysaccharide (Xanthan) that prevented the loss of fats and to the absorption of oil from frying oil medium (Abou-Taleb and Abdel-Razik, 2002). In the same time, the increased ash content may be due to the spices used in coated layer (Yasin and Abou-Taleb, 2007). The evaporation of moisture could be involved in these increments in fat, ash and carbohydrates.

2. Physico-Chemical Quality Criteria for Coated Semi Fried Nile Perch Fish Fillets during Cold Storage (at 4±1°C for 16 Days) as Affected by Tested Essential Oils’ Treatments:

(a) Total volatile basic-nitrogen (TVB-N) content:

The TVB-N is one of the most widely used measurements of seafood quality (LeBlanc and Gill, 1984). Table (2) shows the TVB-N values for the control coated semi fried and flavored, coated semi fried Nile perch fish fillets during cold storage at 4±1°C. As given in Table (2), a significant increasing pattern in TVB-N values was observed in all samples; control and treated samples, during cold storage. The lowest significant incremental rate was recorded in fish fillets sample treated with 1.0 ml of marjoram essential oil per 100g of fish fillets (T2). The highest significant incremental rate was for samples treated with 2 ml of mint essential oil per 100g of fish fillets (T3). The formation of TVB-N is generally indicative with the activity of micro-organisms and the formation tends to be high at high microbial population (Chytiri et al., 2004 and Benjakul et al., 2003). This finding proves the effect of marjoram essential oil on the reduction of the bacterial growth.

Table 2: Changes in total volatile basic-nitrogen (TVB-N) content (mg/100 g wet sample) of flavored, coated semi fried Nile perch fish fillets during cold storage (at 4±1°C for 16 days) as affected by tested essential oils’ treatments.

<table>
<thead>
<tr>
<th>Storage Period</th>
<th>Control</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
<th>T4</th>
<th>T5</th>
<th>T6</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 days</td>
<td>11.8±0.04</td>
<td>12.0±0.06</td>
<td>12.6±0.06</td>
<td>13.1±0.06</td>
<td>13.4±0.06</td>
<td>13.4±0.06</td>
<td>13.4±0.06</td>
</tr>
<tr>
<td>3 days</td>
<td>13.3±0.05</td>
<td>13.4±0.06</td>
<td>13.5±0.06</td>
<td>13.6±0.06</td>
<td>13.6±0.06</td>
<td>13.6±0.06</td>
<td>13.6±0.06</td>
</tr>
<tr>
<td>5 days</td>
<td>14.8±0.06</td>
<td>14.9±0.06</td>
<td>14.9±0.06</td>
<td>14.9±0.06</td>
<td>14.9±0.06</td>
<td>14.9±0.06</td>
<td>14.9±0.06</td>
</tr>
<tr>
<td>7 days</td>
<td>16.2±0.07</td>
<td>16.2±0.07</td>
<td>16.2±0.07</td>
<td>16.2±0.07</td>
<td>16.2±0.07</td>
<td>16.2±0.07</td>
<td>16.2±0.07</td>
</tr>
<tr>
<td>9 days</td>
<td>17.7±0.08</td>
<td>17.7±0.08</td>
<td>17.7±0.08</td>
<td>17.7±0.08</td>
<td>17.7±0.08</td>
<td>17.7±0.08</td>
<td>17.7±0.08</td>
</tr>
<tr>
<td>11 days</td>
<td>19.1±0.09</td>
<td>19.1±0.09</td>
<td>19.1±0.09</td>
<td>19.1±0.09</td>
<td>19.1±0.09</td>
<td>19.1±0.09</td>
<td>19.1±0.09</td>
</tr>
<tr>
<td>13 days</td>
<td>20.5±0.10</td>
<td>20.5±0.10</td>
<td>20.5±0.10</td>
<td>20.5±0.10</td>
<td>20.5±0.10</td>
<td>20.5±0.10</td>
<td>20.5±0.10</td>
</tr>
<tr>
<td>15 days</td>
<td>21.9±0.11</td>
<td>21.9±0.11</td>
<td>21.9±0.11</td>
<td>21.9±0.11</td>
<td>21.9±0.11</td>
<td>21.9±0.11</td>
<td>21.9±0.11</td>
</tr>
</tbody>
</table>

(b) Trimethylamine-nitrogen (TMA-N) content:

The TMA-N is formed from trimethylamine oxide (TMA-O) by microbial action. Data presented in Table (3) shows that the different flavored, coated semi fried fillets had less TMA-N values than the non-treated samples (control).

As illustrated in Table (3), the tested treatments of coated semi fried Nile perch fish fillets with marjoram, mint and thyme essential oils individually caused the reduction of the formation rate of the TMA-N and the prolongation of the shelf-life for fish fillets throughout the cold storage at 4±1°C for 16 days. On the other hand, the TMA-N content in all tested perch fish fillets samples was still within the acceptable European union (EU) limits, of 10-15 mg/100 g (Connell, 1990), that may be due to small amount of the TMA-O. Marine animals are widely known to accumulate trimethylamine oxide (TMAO) in their tissues. This compound occurs at particularly high levels in cod, sharks, rays, skate and squid. In contrast, negligible quantities have been reported in the tissue of freshwater fish (Hebard et al., 1982). However, freshwater fish such as a tilapia (Oreochromis niloticus) and Nile perch (Lates niloticus) caught in Lake Victoria contain relatively high levels of TMAO.
(Anthoni et al., 1990). Also, Chung (2009) reported that the freshwater fish species namely; Micropterus salmoides, Oreochromis niloticus and Siniperca chuatsi, were found to contain TMAO in the range of 510-760, 85-720 and 400-640 mg kg⁻¹, respectively. From the obtained results (Table 3), it could be also observed that treatments with 1 ml of marjoram (T₃) or thyme (T₄) essential oils were the most effective essential oils’ treatments of coated semi fried in delaying the rate of TMA-N increase, as well as in prolonging the shelf-life of coated semi fried Nile perch fish fillets throughout the subsequent cold storage (at – 4 ± 1°C for 16 days). This might be attributed to the effect of these essential oils in reduction of microbial population as mentioned by many studies (Farag et al., 1989; Burt and Reinders, 2003 and Fernandez-Lopez et al., 2006).

### Table 3: Changes in trimethylamine - nitrogen (TMA-N) content (mg /100g wet samples) of flavored, coated semi fried Nile perch fish fillets during cold storage (at 4±1°C for 16 days) as affected by tested essential oils’ treatments.

<table>
<thead>
<tr>
<th>Cold Storage Period</th>
<th>Control</th>
<th>Tested essential oils’ treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T1</td>
<td>T2</td>
</tr>
<tr>
<td>Zero time</td>
<td>1.37±0.01 amused</td>
<td>0.99±0.04 amused</td>
</tr>
<tr>
<td>3 days</td>
<td>2.82±0.02 amused</td>
<td>1.67±0.02 amused</td>
</tr>
<tr>
<td>5 days</td>
<td>4.91±0.02 amused</td>
<td>2.72±0.02 amused</td>
</tr>
<tr>
<td>8 days</td>
<td>7.72±0.03 amused</td>
<td>3.56±0.03 amused</td>
</tr>
<tr>
<td>12 days</td>
<td>Spoiled</td>
<td>4.00±0.03 amused</td>
</tr>
<tr>
<td>16 days</td>
<td>5.23±0.03 amused</td>
<td>4.26±0.04 amused</td>
</tr>
</tbody>
</table>

M±SD*: Mean ± Standard deviation for triplicate determinations of the TMA-N content; the means, within the same row, having different capital superscripts mean that there was a significant difference (p≤0.05) between different treatments in the same storage period; while, the means, within the same column, having different small superscripts mean that there was a significant difference (p≤0.05) between the cold storage periods in the same treatment.

(c) Thiobarbituric acid (TBA) value:

The TBA value is considered as an indicator for the amount of malonaldehyde; the most predominant secondary oxidation products for food lipids, hence it is considered a good chemical constant for quality assurance and for measuring the extent of the secondary oxidation (Autoxidative degradation) of edible lipids during storage, cooking and storage (Schmidt, 1959; Rodriguez-Estrada et al., 1997 and Hassan & Abu-Arab, 2004).

The influence of marjoram, mint and thyme essential oils treatment individually on the TBA values of coated semi fried perch fish fillets during cold storage at 4±1°C for 16 days was investigated. The obtained results are recorded as in Table (4).

<table>
<thead>
<tr>
<th>Cold Storage Period</th>
<th>Control</th>
<th>Tested essential oils’ treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T1</td>
<td>T2</td>
</tr>
<tr>
<td>Zero time</td>
<td>0.47±0.02 amused</td>
<td>0.40±0.02 amused</td>
</tr>
<tr>
<td>3 days</td>
<td>0.56±0.02 amused</td>
<td>0.45±0.01 amused</td>
</tr>
<tr>
<td>5 days</td>
<td>0.91±0.01 amused</td>
<td>0.56±0.02 amused</td>
</tr>
<tr>
<td>8 days</td>
<td>1.26±0.01 amused</td>
<td>0.61±0.03 amused</td>
</tr>
<tr>
<td>12 days</td>
<td>Spoiled</td>
<td>0.69±0.01 amused</td>
</tr>
<tr>
<td>16 days</td>
<td>0.92±0.02 amused</td>
<td>0.82±0.02 amused</td>
</tr>
</tbody>
</table>

M±SD*: Mean ± Standard deviation for triplicate determinations of the TBA value; the means, within the same row, having different capital superscripts mean that there was a significant difference (p≤0.05) between different treatments in the same storage period; while, the means, within the same column, having different small superscripts mean that there was a significant difference (p≤0.05) between the cold storage periods in the same treatment.

(d) Acid value:

The impact of the individual tested essential oils on the acid value of coated semi fried Nile perch fish fillets was studied. The obtained results are recorded as in Table (5).
As shown in Table (5), there were significant differences (p≤0.05) in acid values (as mg KOH per g oil) between treated Nile perch fish fillets samples with marjoram and thyme essential oils and the control untreated fish fillets’ samples at the beginning of cold storage. During the subsequent cold storage, the present results showed that there were a significant (P≤0.05) increases in acid values of different treated fish samples by different rates depending upon the kind and concentration of the essential oil itself, and cold storage period. The highest increasing rate in the acid value during the cold storage was found in the control sample.

From the obtained data (Table 5), it could be also observed that 1% marjoram essential oil treatment (T2) and 1% thyme essential oil treatment (T6) had the highest significant effects against lipid oxidation by lowering acid values till the end of cold storage period; respectively. Similarly, Yasin and Abou -Taleb (2007) found that the marjoram spice had higher effect on acid values than thyme spice in mullet fillets. Their effects are due to its content of phenol compounds, which having an important role in the stabilization of lipid oxidation, antihypertensive and antithrombic effects, and reduce carcinostatic properties (Jang et al., 1997).

### Table 5: Changes in acid value of flavored, coated semi fried Nile perch fillet samples during cold storage (at 4 ±1ºC for 16 days) as affected by tested essential oils’ treatments.

<table>
<thead>
<tr>
<th>Cold Storage Period</th>
<th>Control</th>
<th>Tested essential oils’ treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T1</td>
<td>T2</td>
</tr>
<tr>
<td>Zero time</td>
<td>0.83±0.01**</td>
<td>0.71±0.01**</td>
</tr>
<tr>
<td>3 days</td>
<td>2.69±0.00**</td>
<td>2.23±0.01**</td>
</tr>
<tr>
<td>5 days</td>
<td>5.63±0.49**</td>
<td>3.21±0.01**</td>
</tr>
<tr>
<td>8 days</td>
<td>6.56±0.33**</td>
<td>4.22±0.04**</td>
</tr>
<tr>
<td>12 days</td>
<td>Spoiled</td>
<td>4.15±0.01**</td>
</tr>
<tr>
<td>16 days</td>
<td>5.58±0.33**</td>
<td>4.22±0.04**</td>
</tr>
</tbody>
</table>

**Mean ± Standard deviation for triplicate determinations of acid value; the means, within the same row, having different capital superscripts mean that there was a significant difference (p≤0.05) between different treatments in the same storage period, while the means, within the same column, having different small superscripts mean that there was a significant difference (p≤0.05) between the cold storage periods in the same treatment.

3. Microbial Quality Criteria for Coated Semi Fried Nile Perch Fish Fillets during Cold Storage (at 4±1ºC for 16 Days) as Affected by Tested Essential Oils’ Treatments:

(a) Total viable Count (TVC):

The changes in the total viable count of flavored, coated semi fried fish fillets during cold storage are shown in Fig. (1). As given in Figure (1), all marjoram, mint and thyme essential oils treatments caused a significant (P≤0.05) reduction in microbial count of treated-fish fillets immediately at zero time except T3 at which the sample was treated with 1.5 ml mint essential oil per 100g of fish fillets. The TVC of the flavored coated semi fried Nile perch fillets increased significantly (P≤0.05) during storage at 4±1ºC, but they did not exceed the maximum acceptable count for freshwater fish which is 10^7 cfu g^-1, as recommended by ICMSF (1978) and FAO (2005). At the point of rejection, total viable count of Nile perch fillets in ice was 8.0 log cfu/g (FAO, 2005). While, Gram et al., (1989) found that the total viable count was 10^7 cfu/g before the fish was rejected in ice-stored Nile perch. The current results (Fig.1) also illustrated that the fish samples treated with either 1.0 ml marjoram essential oil per 100g fish fillets (T2) or with 1.0 ml thyme essential oil per 100g fish fillets (T6) showed the lowest bacterial count. In this rank, Deans and Ritchie (1987) reported that the marjoram oil, among fifty essential oils that may be useful as antimicrobial agents against 25 microorganisms, may have the greatest potential for use in industrial applications. The antimicrobial activity of these essential oils could be resulted from the hydrophobic nature which enables them to partition in the lipids of the bacteria cell membrane and mitochondria, disturbing the structures and rendering it more permeable (Sikkema et al., 1994).

(b) Psychrotrophic bacteria count:

The psychrotrophic bacteria are well known as the main cause of spoilage of refrigerated fish. Therefore, the inhibitory effect of marjoram, mint and thyme essential oils on psychrotrophic bacteria growth in coated semi fried fish fillets during cold storage (at 4±1ºC for 16 days) was determined. The obtained results are represented as in Figure (2).

As shown in Fig. (2), control untreated fish fillets sample with any tested essential oil had the highest count of psychrotrophic bacteria along cold storage period compared to other treatments. From the same Fig., it could be detected that both thyme and marjoram essential oils have strong inhibitory effects against the growth of psychrotrophic bacteria which reduced significantly (P≤0.05) immediately at zero time from 3.97 log cfu g^-1 for the control sample to 3.88, 3.65, 3.89, 3.75 and 3.93 log cfu g^-1 for fish fillets samples treated with 0.5 and 1.0 % marjoram essential oil (T1 & T2), 0.5 and 1.0 % thyme essential oil (T3 & T4), and 2.0 % mint essential oil (T6); respectively. All different treatments significantly (P≤0.05) reduced psychrotrophic bacterium count during...
cold storage period. In this rank, Mejholm and Dalgaard (2002) found that oregano essential oil (0.05%, v/w) reduced growth of *Photobacterium phosphoreum* in naturally contaminated cod fillets kept under modified atmosphere packages.

![Graph](image)

**Fig. 1:** The changes in total viable count (Log cfu g⁻¹) of flavored, coated semi fried Nile perch fish samples during cold storage (at 4±1°C for 16 days) as affected by tested essential oils’ treatments.

Control: untreated fish fillets sample with any tested essential oil; T₁: 0.5 ml of marjoram essential oil per 100g of fish; T₂: 1.0 ml of marjoram essential oil per 100g of fish; T₃: 1.5 ml mint essential oil per 100g of fish; T₄: 2.0 ml mint essential oil per 100g of fish; T₅: 0.5 ml thyme essential oil per 100g of fish; T₆: 1.0 ml thyme essential oil per 100g of fish.

![Graph](image)

**Fig. 2:** The changes in Psychrotrophic bacteria count (Log cfu g⁻¹) of flavored, coated semi fried Nile perch fish samples during cold storage (at 4±1°C for 16 days) as affected by tested essential oils’ treatments.

Control: untreated fish fillets sample with any tested essential oil; T₁: 0.5 ml of marjoram essential oil per 100g of fish; T₂: 1.0 ml of marjoram essential oil per 100g of fish; T₃: 1.5 ml mint essential oil per 100g of fish; T₄: 2.0 ml mint essential oil per 100g of fish; T₅: 0.5 ml thyme essential oil per 100g of fish; T₆: 1.0 ml thyme essential oil per 100g of fish.

(c) *Pseudomonas* spp. bacteria count:

It is well known that fish microflora includes bacterial species such as *Pseudomonas, Alcaligenes, Vibrio, Serratia* and *Micrococcus* (Gram and Huss, 2000). Changes in the *Pseudomonas* of flavored, coated semi fried fish fillets during cold storage (at 4±1°C for 16 days) are shown in Fig. (3).

As illustrated in Fig. (3), all treatments, except T₃ (1.5 ml of mint essential oil per 100 g of fish), gave a significant reduction (P≤0.05) in the *Pseudomonas* spp. bacterium count immediately after treatment, as compared with the control fish sample. The *Pseudomonas* spp. counts in flavored coated semi fried Nile perch fillet samples decreased through the first three days of cold storage for samples treated with either 1.0 ml marjoram essential oil per 100g fish fillets (T₂) or with 1.0 ml thyme essential oil per 100g fish fillets (T₆) and
then they gradually increased during cold storage. From the present results, it could be reported that marjoram, mint, and thyme essential oils had antimicrobial activity on pseudomonas bacterium species. In this concern, Erkan et al. (2011) found that the addition of 1% thyme and 1% laurel essential oils have effect on reduction of the *Pseudomonas* spp. counts in bluefish samples compared with control samples stored in ice which reached 4.86, 3.95 and 6.49 log cfu g\(^{-1}\) after 11 days of ice storage.

| Fig. 3: | The changes in *Pseudomonas* spp. count (Log cfu g\(^{-1}\)) of flavored, coated semi fried Nile perch fish samples during cold storage (at 4±1°C for 16 days) as affected by tested essential oils’ treatments. Control: untreated fish fillets sample with any tested essential oil; T1: 0.5 ml of marjoram essential oil per 100g of fish; T2: 1.0 ml of marjoram essential oil per 100g of fish; T3: 1.5 ml mint essential oil per 100g of fish; T4: 2.0 ml mint essential oil per 100g of fish; T5: 0.5 ml thyme essential oil per 100g of fish; T6: 1.0 ml thyme essential oil per 100g of fish. |

(d) Yeasts and molds count: The changes in the count of yeasts and molds of flavored, coated semi fried Nile perch fillets during cold storage at 4 ±1°C as affected by treating with marjoram, mint and thyme essential oils individually are shown in Fig. (4).

| Fig. 4: | The changes in Yeasts and molds count (Log cfu g\(^{-1}\)) of flavored, coated semi fried Nile perch fish samples during cold storage (at 4±1°C for 16 days) as affected by tested essential oils’ treatments. Control: untreated fish fillets sample with any tested essential oil; T1: 0.5 ml of marjoram essential oil per 100g of fish; T2: 1.0 ml of marjoram essential oil per 100g of fish; T3: 1.5 ml mint essential oil per 100g of fish; T4: 2.0 ml mint essential oil per 100g of fish; T5: 0.5 ml thyme essential oil per 100g of fish; T6: 1.0 ml thyme essential oil per 100g of fish. |
From the obtained results (Fig. 4), it could be noticed that the initial count of yeasts and molds was significantly reduced (p≤0.05) immediately after treating the fish fillets samples by 1.0 ml marjoram essential oil (T1), 1.0 ml thyme essential oil (T2), 0.5 ml marjoram essential oil (T3), 0.5 ml thyme essential oil (T4), and 2.0 ml mint essential oil (T5) and 1.5 ml mint essential oil (T6) per 100 g wet fish fillets, since they were: 1.36, 1.46, 1.53, 1.66, 1.78, and 1.80 log cfu g⁻¹, compared with the control sample (1.89 cfu g⁻¹); respectively. The yeast and mold counts of the flavored, coated semi fried Nile perch fillets increased during storage at 4±1°C until reached to 1.75 log cfu g⁻¹ in fillets treated with 1.0 ml marjoram essential oil/100 g fish fillets (T1). The current results are in accordance with those obtained by Kassem et al. (2011) who found that the 0.04 and 0.06% thyme essential oil treated beef burger samples revealed a marked reduction in total mould counts than the control sample, at 12th day of storage.

4. Sensory Quality Properties for Coated Semi Fried Nile Perch Fish Fillets during Storage (at 4±1°C for 16 Days) as Affected by Tested Essential Oils’ Treatments:

Data in Table (6) revealed the sensory properties score of control and the different coated semi fried Nile perch fillets treated with tested essential oils individually during cold storage (at 4±1°C for 16 days).

For appearance evaluation, as shown in Table (6), there were significant (p≤0.05) differences between control and treated samples before storage were observed. Whereas, the highest score (8.93) was found for the control fish fillet sample. During cold storage of coated semi fried Nile perch fillets, the samples treated with marjoram and thyme essential oils did not reach the limited acceptable score at 16th day. As shown from color results (Table 6), there was no significant (p≤0.05) difference in color scores was found between all tested fish fillet samples at either initial zero time or the end of cold storage the groups. Where, the color scores at the end of cold storage period (after 16 days), were 6.64, 7.02, 6.23, 6.44, and 6.92 for fish samples treated with 0.5 ml marjoram oil (T1), 1.0 ml marjoram oil (T2), 2.0 ml mint oil (T3), 0.5 ml thyme oil (T4) and 1.0 thyme oil per 100 grams wet fish fillets; respectively. While, fish fillets sample treated with 1.5 ml mint oil / 100 g fish fillets (T5) and the control untreated fish fillets were sensory spoiled.

Table 6: Sensory Quality properties of flavored, coated semi fried Nile perch fish samples stored under the cold storage conditions (at 4±1°C for 16 days) as affected by tested essential oils’ treatments.

<table>
<thead>
<tr>
<th>Cold Storage Period</th>
<th>Appearance</th>
<th>Zero time</th>
<th>16 day</th>
<th>Color</th>
<th>Zero time</th>
<th>16 day</th>
<th>Odor</th>
<th>Zero time</th>
<th>16 day</th>
<th>Taste</th>
<th>Zero time</th>
<th>16 day</th>
<th>Odor</th>
<th>Zero time</th>
<th>16 day</th>
<th>Tenderness</th>
<th>Zero time</th>
<th>16 day</th>
<th>Juiciness</th>
<th>Zero time</th>
<th>16 day</th>
<th>Overall acceptability</th>
<th>Zero time</th>
<th>16 day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8.54±0.26</td>
<td>8.36±0.50</td>
<td>8.43±0.38</td>
<td>8.84±0.14</td>
<td>8.97±0.06</td>
<td>8.96±0.07</td>
<td>8.92±0.07</td>
<td>8.73±0.36</td>
<td>8.87±0.21</td>
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<td>T1</td>
<td>8.91±0.07</td>
<td>8.51±0.63</td>
<td>8.84±0.14</td>
<td>8.97±0.06</td>
<td>8.96±0.07</td>
<td>8.92±0.07</td>
<td>8.73±0.36</td>
<td>8.87±0.21</td>
<td>8.63±0.45</td>
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<td>T2</td>
<td>5.70±0.75</td>
<td>5.96±0.07</td>
<td>5.81±0.02</td>
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<td>T3</td>
<td>5.60±0.02</td>
<td>5.12±0.74</td>
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<td>T4</td>
<td>8.73±0.36</td>
<td>8.87±0.21</td>
<td>8.64±0.44</td>
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<td>T5</td>
<td>7.00±0.20</td>
<td>6.92±0.19</td>
<td>6.44±0.44</td>
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<tr>
<td>T6</td>
<td>6.22±0.15</td>
<td>6.63±0.54</td>
<td>6.63±0.54</td>
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From the obtained results (Table 6), it could be also observed that the odor scores of coated semi fried fish fillets exhibited the same trend of their color scores during cold storage. Then, there were no significant differences in the odor scores between the all tested fish fillets either after the first day (initial zero time) or after 16 days of cold storage. Whereas, the odor scores at the end of cold storage period (after 16 days) were 6.44, 6.81, 5.96, 6.23 and 6.63 for fish fillets treated with T1, T2, T3, T4, and T6, respectively, while those treated with T1 and the control were sensory spoiled. Acceptability scores for taste of the control and treated coated semi fried Nile Perch fillets’ samples decreased with extending the cold storage period. The acceptability limit of taste for 2.0 ml mint essential oil (T2) treated sample was reached (5.74), while the control and 1.5 ml mint essential oil (T3) treated sample were spoiled after 16 days of cold storage. With regard the tenderness property, at the initial zero time, there was no significant (p≤0.05) difference in tenderness scores between the all tested fish fillets’ samples. During storage there was a significant decrease pattern in tenderness scores of all tested fish fillets with prolonging the cold storage period. The 1.0 ml marjoram essential oil treated sample showed the significant highest tenderness score (6.83) after 16 day and 2.0 ml mint essential oil treated sample showed a significant lowest score (5.44) at the end of cold storage period. Concerning the juiciness property, there was no significantly (p≤0.05) difference in juiciness scores at the initial zero time of cold storage between all tested fish.
samples. After 16 days of cold storage, 1.0 ml marjoram essential oil (T6) treated fish sample showed the highest juiciness mean score (6.72) and 1.5 ml mint essential oil (T4) treated fish sample showed the lowest mean score (5.88). From overall acceptability results (Table 6), there was no statistically significant difference between all coated semi fried fish fillets’ samples at the initial zero time. After 16 days of cold storage, 1.0 ml marjoram essential oil treated sample showed the significant (p≤0.05) highest mean score of overall acceptability and 2.0 ml mint essential oil treated sample showed the significant (p≤0.05) lowest mean score. In this concern, Gram and Huss (1996) reported that the NPN fraction of the fish flesh consists of low-molecular-weight water-soluble nitrogen contains compounds, particularly free amino acids and nucleotides, that allow it to serve as a readily available bacterial growth substrate. Decomposition of these compounds is responsible for many of the off-odors and off-flavors typically found in spoilage. For example, the breakdown of cysteine and methionine by certain microbes, both sulfur-containing amino acids, forms hydrogen sulfides and methylmercaptane respectively which causes undesirable odors to emanate from spoiled fish.

**Conclusion and Recommendation:**

In general, the tested essential oils’ treatments caused a significant extending of the shelf-life, as well as enhancement the physico-chemical, microbiological and sensory quality attributes for coated semi fried Nile perch fish fillets during cold storage. In addition, fish samples treated with 1.0 ml marjoram essential oil (T2) followed by samples treated with 1.0 ml thyme essential oil (T6) attained the highest scores for sensory quality attributes and exhibited the better acceptability than the control and other treated fish samples. Therefore, the present findings recommended that the essential oils of marjoram, mint and thyme should be utilized for extending the shelf-life and enhancing quality attributes of coated semi fried fish fillets during cold storage.

**References**


