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Antioxidant and Antimicrobial Effects of Marjoram and Thyme in Coated Refrigerated Semi Fried Mullet Fish Fillets

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Abstract: This study was carried out to investigate the effect of marjoram and thyme on the quality of semi fried mullet fish fillets during cold storage. Mullet fillets were divided into five batches, one batch dipped into the edible coating solution (wheat flour, sodium chloride, cumin and xanthan) and recorded as control sample. To evaluate the effect of different antioxidant and antimicrobial sources, thyme and marjoram 2.5 and 5.0% of the tested materials were mixed with edible coating solution. The second and third fish fillet batches were dipped into edible coating solutions containing 2.5 and 5.0% thyme respectively, while the fourth and five batches dipped into edible coating solutions containing 2.5 and 5.0% of marjoram respectively. Then chemical [total volatile basic nitrogen, trimethylamine nitrogen, thiobarbituric acid, acid value and peroxide value], microbiological [total bacterial counts, psychrophilic bacterial counts and enterobacteriaceae counts] and sensory analyses were used to evaluate the preservative effect of this materials during storage at 4°C. Significant incremental pattern was observed in TVB-N and TMA-N values in all samples during subsequent cold storage. The lowest significant incremental rate was recorded in samples coated with 5% marjoram. The results showed that there was a significant ($p<0.01$) increase in acid values in different treatments during storage by different rates. Samples coated with 5% thyme showed the lowest rate of peroxide formation. TBC counts were gradually increased during cold storage for all samples with different ratios depending on the concentration of spices. Regarding to the different treatments both coated samples with thyme at 2.5 or 5% showed the lowest incremental pattern in psychrophilic bacterial counts at any time of the cold storage. Thyme and marjoram have strong effects against the growth of enterobacteriaceae at both concentrations (2.5 and 5%). There was a significant ($p<0.01$) enhancement in sensory attributes which appeared in samples containing marjoram and thyme at both concentrations (2.5 and 5%) compared to control sample. Generally, samples contained 5% marjoram followed by samples contained 5% thyme attained the highest scores than other treatments.

Keywords: Mullet · marjoram. thyme · antioxidants · antimicrobials · spices · fish fillets

INTRODUCTION

Oxidation of lipids which occurs during raw material storage, processing, heat treatment and further storage of final products is one of the basic processes causing rancidity in food products leading to their deterioration. Taste and aroma of a product can be the criteria for rejection of any kind of food. Products of lipid oxidation influence other food constituents, e.g. they interfere with the absorption of protein [1]. Mainly synthetic antioxidants are used in order to prolong the storage stability of food. However, toxicologists and nutritionists have for long noted the noxiousness of some synthetic antioxidants such as BHA and BHT which are used in food processing. Therefore, the development of effective natural antioxidants has been investigated to retard lipid oxidation [2] Consumers generally perceive natural antioxidants as better than synthetic additives. Phenols are one of the most important groups of natural antioxidants. They occur only in material of plant origin and they are known to protect easily-oxidizable constitutes of food from oxidation. Especially worthy of notice are spices and herbs which for many years have been used as additives to enhance the sensory features

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of food [3]. As recognized by [4], the spices and herbs may fulfill more than one function in food to which they are added. In addition to imparting flavor, certain spices prolong the storage life of food by a bactriostatic or bactericidal activity and some prevent rancidity by their antioxidant activity.

The leafy part of a plant belong to the *Lamiaceae* family has been added to meat, fish and food products. In addition to improving flavor certain spices prolong the storage life of food by an antimicrobial activity as found by [5]. Marjoram (*Origanum majorana* L.) and thyme (*Thymus vulgaris* L.) are used as spices and condiments. The main component of their essential oils was carvacrol which represented at 81.5 and 45.1% in marjoram and thyme, respectively [6]. The dill, marjoram and geranium oils 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 [7]. Also, these oils have strong antimicrobial properties towards fungal and bacterial populations. As reported by [8], the ethanolic extract of marjoram showed antimicrobial activity against food borne strains of bacteria and fungi. In the same field [9] found that the essential oil of thyme has a significant rate of antifungal and antibacterial activities with strongly inhibited lipid peroxidation and high OH radical scavenging.

This study was carried out to investigate the effect of marjoram and thyme on the quality of semi fried mullet fish fillets during cold storage.

MATERIALS AND METHODS

Fresh mullet fish of the species *Mugil capito* were obtained from El-Sarwo farm, El-Dakahlia Governorate, National Institute of Oceanography and Fisheries, during 7/2006 season. The mullet fish were approximately 500-600 g. weight each. The fish samples were put in ice box and transferred to Food Science Department, Faculty of Agriculture, Ain Shams University for further technological treatments and analysis. Coating agents including wheat flour, sodium chloride, cumin, thyme and marjoram were obtained from local market, where xanthan (bacterial source) was obtained from Sigma Chemical Co. (St. Louis, Mo.), USA.

Fish samples were washed, gutted, filleted, rewashed and drained. Fillets were divided into five batches. One batch was dipped into the edible coating solution (wheat flour, sodium chloride, cumin and xanthan) and recorded as control sample. To evaluate the effects of investigated antioxidant and antimicrobial sources, thyme and marjoram 2.5 and 5.0% materials were mixed with edible coating solution. The second and third fish fillet batches were dipped into edible coating solutions containing 2.5 and 5.0% thyme respectively, where the fourth and five batches were dipped into edible coating solutions containing 2.5 and 5.0% marjoram respectively.

All fish fillets batches were left four 3-4 minutes at room temperature and then semi-fried using an electrical fryer pan (Moulinex brand) in sunflower oil heated at 170°C for 1 minute, then drained in basket to remove excess oil. Different coated fish fillet samples were packaged in polyethylene bags and stored for 16 days at 4°C±1.

Moisture, crud protein, fat and ash contents and trimethylamine-nitrogen (TMA-N) as well as peroxide value were determined as described in [10]. Total volatile basic nitrogen (TVB-N), acid value and thiobarbituric acid reactive substances (TBARS) were determined according to [11]. Total aerobic bacterial counts and aerobic plate counts of psychrotrophic bacteria were determined by spread plating on plate count agar, employing an incubation condition at 37°C for 24-48 h and 7°C for 10 days, respectively. Enterobacteriaceae counts were determined by using vilot red bile agar and incubation condition at 37°C for 24-48 h. Count of total aerobic bacteria, psychrotrophic bacteria and enterobacteriace were expressed as colony-forming units (cfu) gm^{-1}. Sensory attributes of the prepared coated mullet fish fillets were determined after frying in corn oil at 170°C for 5 minutes by a panel group of ten members, randomly selected from the staff members of the Dept. of Food Sci., Fac. of Agric., Ain Shams Univ. Panelists were asked to evaluate appearance, color, odor, taste, tenderness, juiciness and overall acceptability according to 9-point hedonic scale [12]. The statistical analysis [13] was used to carry out mean values, standard division in addition to an over all analysis of variance (ANOVA) and Least Significant Differences (LSD) at 0.01 level.

RESULTS

Proximate chemical composition: Mean values for the proximate chemical composition of raw and semi fried mullet fish fillets are given in Table 1. For raw fillets the moisture, protein, fat, ash and free nitrogen extract were 72.53, 81.15, 9.28, 5.67 and 3.9% (on dry basis), respectively. Meanwhile, the corresponding values in semi fried cooked fillets were 45.65, 69.32, 15.72, 6.18 and 8.78%, respectively. From these results it could be observed that, semi fried process increased the moisture and protein losses by 37.06 and 14.58%, respectively.

Table 1: Proximate composition of raw and coated semi fried mullet fish fillets (on dry weight basis)

	Proximate composition (%)				
Fish fillets	Moisture	Protein	Fat	Ash	FNE*
Raw fillets	72.53±0.23	81.15±0.35	9.28±0.15	5.67±0.28	3.90±0.18
Coated semi fried fillets	45.65±0.27	69.32±0.15	15.72±0.32	6.18±0.35	8.78±0.18

*FNE: Free nitrogen extract calculated by differences

Table 2: Total volatile basic nitrogen of coated semi fried mullet fillet samples stored at 4°C±1 (mg/100 g wet samples)

	Coated semi fried mullet fillets				
Storage period (days)	Control	2.5%Thyme	5.0%Thyme	2.5%Marjoram	5.0%Marjoram
Zero	11.70±0.8 ^{aC}	10.40±0.7 ^{aD}	10.15±2.0 ^{aC}	10.70±0.8 ^{aC}	10.65±1.0 ^{aE}
4	18.10±2.0 ^{aB}	15.90±0.8 ^{bC}	15.50±0.8 ^{bB}	16.50±0.7 ^{abB}	15.45±0.7 ^{bD}
6	21.00±0.9 ^{aA}*	18.20±0.7 ^{bB}	16.75±0.5 ^{bB}	17.50±0.7 ^{bB}	16.60±0.7 ^{bC}
8		20.00±0.8 ^{aA}*	18.80±0.6 ^{Ba}	20.30±0.8 ^{aA}*	18.20±0.7 ^{bCB}
12			20.50±0.7 ^{aA}*		18.75±0.4 ^{bAB}
16					20.20±0.2 ^{aA}*

*Onset of spoilage, Different superscript small characters mean significant differences between different treatments in the same storage periods ($p \leq 0.05$), Different superscript capital characters mean significant differences between different storage periods in the same treatment ($p \leq 0.01$)

Table 3: Trimethylamine-nitrogen of coated semi fried mullet fillet samples stored at 4°C±1 (mg/100 g wet samples)

	Semi fried mullet fillets coated				
Storage period (days)	Control	2.5%Thyme	5.0%Thyme	2.5%Marjoram	5.0%Marjoram
Zero	1.50±0.1 ^{aC}	1.30±0.1 ^{aD}	0.85±0.1 ^{bD}	1.35±0.03 ^{aD}	0.84±0.03 ^{bF}
4	4.38±0.6 ^{aB}	2.27±0.1 ^{bcC}	1.71±0.1 ^{cC}	2.59±0.2 ^{bC}	1.65±0.3 ^{cE}
6	6.83±0.4 ^{aA}*	4.67±0.5 ^{cB}	2.50±0.3 ^{dC}	5.12±0.3 ^{bB}	2.79±0.3 ^{dD}
8		6.73±0.3 ^{aA}*	4.20±0.5 ^{bB}	6.97±0.4 ^{aA}*	3.56±0.3 ^{bC}
12			5.90±0.3 ^{aA}*		4.35±0.1 ^{bB}
16					5.98±0.4 ^{aA}*

*Onset of spoilage, Different superscript small characters mean significant differences between different treatments in the same storage periods ($p \leq 0.05$), Different superscript capital characters mean significant differences between different storage periods in the same treatment ($p \leq 0.01$)

Total volatile basis nitrogen (TVB-N): The mean values of TVB-N are summarized in Table 2, which estimated the degree of fish deterioration during storage period. In the beginning of cold storage period the TVB-N of fish fillets (control and treatments) ranged from 10.15 to 11.7 mg per 100 g wet sample, while as the period of cold storage at 4°C±1 increased the TVB-N values increased as shown in Table 2 for all fish fillet samples with different rates depending on the nature of treatments. As shown in Table 2, the treatment with 5% thyme and marjoram was more effective in delaying the rate of TVB-N increase during the subsequent cold storage.

Trimethylamine-nitrogen (TMA-N): Table 3 shows TMA-N values of raw and semi fried coated mullet fish fillets during cold storage. Significant incremental pattern in TMA-N values was observed in all samples during subsequent cold storage. The lowest significant incremental rate was recorded in samples coated with 5% marjoram. In general, as the concentration of thyme and marjoram increase the TMA-N values decrease.

Thiobarbituric acid values (TBA) as O.D.: The evolution of TBA values (O.D.) of raw and semi fried coated mullet fish fillets during cold storage are shown in Table 4. In the first day, there was a significant effect of frying method on TBA values of different samples but the lowest effect of this method was apparent in samples coated with 5% thyme and marjoram. During cold storage there was an incremental pattern in TBA values in different samples which indicated oxidation in mullet fish fillets.

Table 4: Thiobarbituric acid values of coated semi fried mullet fillet samples stored at 4°C±1 (as OD at 538 nm)

	Semi fried mullet fillets coated				
Storage period (days)	Control	2.5%Thyme	5.0%Thyme	2.5%Marjoram	5.0%Marjoram
Zero	1.504±0.1 aC	1.175±0.03 bB	0.853±0.03 dB	1.053±0.03 cD	0.863±0.03 dF
4	1.803±0.04 aB	1.217±0.04 bB	0.902±0.04 cB	1.177±0.02 bC	0.971±0.03 cE
6	1.899±0.04 aA*	1.482±0.04 bA	0.970±0.02 eB	1.295±0.02 cB	1.097±0.1 dD
8		1.565±0.1 aA*	1.042±0.1 cB	1.475±0.05 abA*	1.377±0.04 bC
12			1.923±0.1 aA*		1.482±0.04 bB
16					1.966±0.01 aA*

*Onset of spoilage, Different superscript small characters mean significant differences between different treatments in the same storage periods ($p \leq 0.05$), Different superscript capital characters mean significant differences between different storage periods in the same treatment ($p \leq 0.01$)

Table 5: Acid values of coated semi fried mullet fillet samples stored at 4°C±1 (as mg KOH alcohol g^{-1} oil)

	Semi fried mullet fillets coated				
Storage period (days)	Controlf	2.5%Thyme	5.0%Thyme	2.5%Marjoram	5.0%Marjoram
Zero	1.77±0.1 aC	1.69±0.04 abD	1.61±0.1 cbE	1.53±0.1 cD	1.58±0.1 cbF
4	4.75±0.5 aB	2.81±0.3 bcC	2.47±0.3 bcD	2.89±0.2 bC	2.25±0.2 cE
6	6.91±0.2 aA*	4.91±0.2 bB	4.43±0.3 cC	3.99±0.1 dB	3.60±0.3 eD
8		7.16±0.3 aA*	5.84±0.3 bB	6.71±0.4 aA*	4.02±0.2 cC
12			6.51±0.4 aA*		5.04±0.2 bB
16					6.44±0.3 aA*

*Onset of spoilage, Different superscript small characters mean significant differences between different treatments in the same storage periods ($p \leq 0.05$), Different superscript capital characters mean significant differences between different storage periods in the same treatment ($p \leq 0.01$)

Table 6: Peroxide values of coated semi fried mullet fillet samples stored at 4°C±1 (as meq Kg^{-1} oil)

	Semi fried mullet fillets coated				
Storage period (days)	Control	2.5%Thyme	5.0%Thyme	2.5%Marjoram	5.0%Marjoram
Zero	4.91±0.2 aC	4.50±0.04 bD	3.91±0.1 cE	4.06±0.1 cD	3.93±0.1 cF
4	11.50±0.9 aB	8.95±0.3 bC	7.53±0.3 dD	8.51±0.2 bcC	8.04±0.2 cdE
6	21.27±1.0 aA*	16.01±0.2 bB	11.17±0.3 cC	15.55±0.1 bB	12.87±0.3 cD
8		21.28±0.3 aA*	18.13±0.3 bB	21.87±0.4 aA*	18.67±0.2 bC
12			22.23±0.4 aA*		20.92±0.2 bB
16					22.47±0.3 aA*

*Onset of spoilage, Different superscript small characters mean significant differences between different treatments in the same storage periods ($p \leq 0.05$), Different superscript capital characters mean significant differences between different storage periods in the same treatment ($p \leq 0.01$)

Acid value as (mg KOH alcohol per g oil): The differences in mean acid value between different treatments and control fillets samples were significant ($p<0.01$) from the beginning of cold storage and during the subsequent cold storage. The results showed that there was a significant ($p<0.01$) increase in acid values in different treatments during storage by different rates. The highest incremental rate was found in the control sample Table 5. The concentrations of marjoram at 2.5 and 5% showed the highest significant effects on lipid oxidation by lowering acid values than thyme at the same concentrations till the end of cold storage.

Peroxide value (meq per Kg oil): As appears to Table 6, at the beginning of storage the peroxide values of different treatments were high. The samples contained in its coat 5% thyme showed the lowest rate of peroxide formation. Samples can be arranged in ascending order as follows: samples contained 5% thyme, 5% marjoram, 2.5% thyme and 2.5% marjoram. The highest values were found in control sample during any time of storage.

Total Bacterial Counts (TBC): Total Bacterial Counts (TBC) were enumerated for different samples during the cold storage as given in Fig. 1. At zero time of storage,

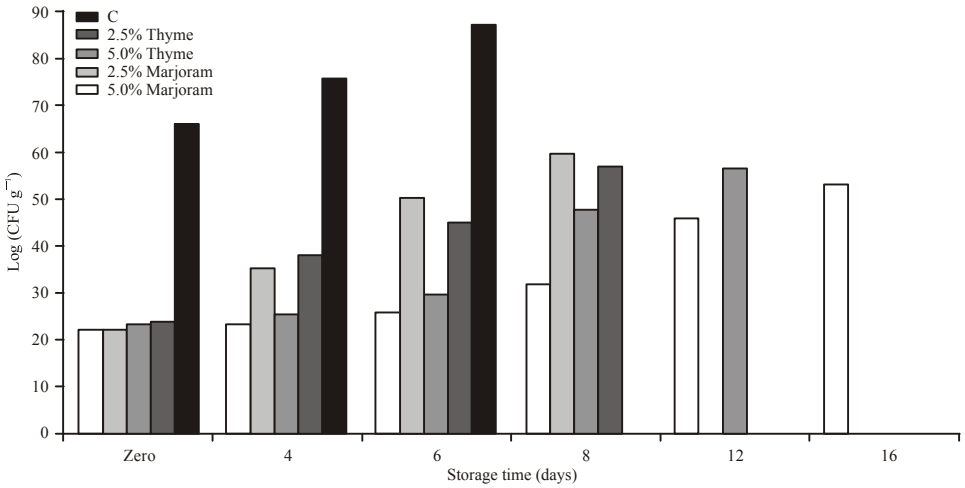

Fig. 1: Total bacterial counts (CFU g^{-1}) of coated semi fried mullet fish samples during cold storage

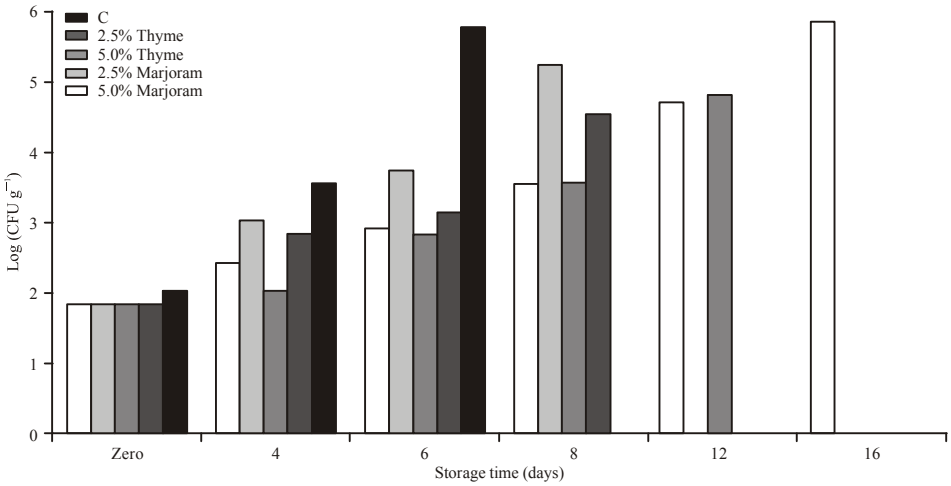

Fig. 2: Psycchrophilic bacterial counts (CFU g^{-1}) of coated semi fried mullet fish samples during cold storage

control samples showed the highest mean counts comparing to other samples contained thyme or marjoram with different concentrations. TBC counts were gradually increased during cold storage for all samples with different ratios depending on the concentration of spices. The incremental pattern in TBC can be arranged in an ascending order as follows: samples coated with 5% marjoram, 5% thyme, 2.5% thyme and finally 2.5% marjoram. In general, as the concentration of spices increase the TBC numbers increased with the same spice material.

Psychrophilic bacterial counts: Data presented in Fig. 2 show psycchrophilic bacterial counts of different coated mullet fish fillets during cold storage. As demonstrated by the different treatments both coated samples with thyme at 2.5 and 5% showed the lowest incremental pattern in this parameter at any time of cold storage.

Enterobacteriaceae counts: As shown in Fig. 3, it could be observed that control sample had the highest counts of enterobacteriaceae at any time of cold storage compared to other treatments. From the same Fig it could be easily to say that, both thyme and marjoram have strong effects against the growth of enterobacteriaceae at both concentrations 2.5 and 5%. As the concentration of marjoram and thyme increase the counts of enterobacteriaceae reduce especially after 4, 6 and 8 days of cold storage.

Sensory evaluation: Data in Table 7 revealed that the sensory properties of different treated semi fried mullet

Table 7: Sensory properties of coated semi fried mullet fillet samples stored at 4°C±1

	Semi fried mullet fillet coated				
Storage period (days)	Control	2.5% thyme	5.0% thyme	2.5% marjoram	5.0% marjoram
Appearance					
Zero	8.67^{aA}	8.67^{aA}	8.67^{aA}	8.67^{aA}	8.67^{aA}
4	6.67^{cB}	7.67^{aB}	7.33^{bB}	7.33^{bB}	8.00^{aA}
6	5.00^{cB*}	6.66^{bC}	7.00^{aB}	6.67^{bC}	7.33^{aB}
8		5.55^{cD*}	6.67^{bC}	5.00^{dD*}	6.33^{aC}
12			5.67^{aD*}		6.00^{aD}
16					5.00^{E*}
Color					
Zero	8.67^{aA}	8.67^{aA}	8.67^{aA}	8.67^{aA}	8.67^{aA}
4	6.67^{bB}	7.67^{aB}	7.33^{aB}	7.33^{aB}	8.00^{aA}
6	5.00^{bC*}	6.66^{aC}	7.00^{aB}	6.67^{aB}	7.33^{aB}
8		5.55^{cD*}	6.67^{bC}	5.00^{cC*}	6.33^{aC}
12			5.67^{bD*}		6.00^{aC}
16					5.00^{D*}
Odor					
Zero	8.00^{bA}	8.55^{aA}	8.67^{aA}	9.00^{aA}	9.55^{aA}
4	7.67^{bB}	7.67^{bB}	8.00^{aA}	8.55^{aA}	9.00^{aA}
6	5.55^{cC*}	6.66^{cC}	7.55^{bB}	7.55^{bB}	8.53^{aB}
8		5.55^{cD*}	6.67^{bC}	6.00^{bC*}	8.00^{aB}
12			5.67^{bD*}		7.50^{aB}
16					6.50^{B*}
Taste					
Zero	7.85^{bA}	8.67^{aA}	8.67^{aA}	8.67^{aA}	9.67^{aA}
4	6.67^{cB}	7.00^{bB}	8.33^{aA}	7.55^{bB}	9.00^{aA}
6	5.00^{cC*}	6.55^{bC}	7.00^{bB}	6.55^{bC}	8.50^{aB}
8		5.55^{cD*}	6.67^{bB}	6.00^{bC*}	7.55^{aC}
12			5.00^{bC*}		6.50^{aD}
16					6.00^{D*}
Tenderness					
Zero	8.00^{aA}	8.55^{aA}	8.67^{aA}	8.67^{aA}	8.67^{aA}
4	7.55^{aA}	7.67^{aA}	8.00^{aA}	7.55^{aB}	8.00^{aA}
6	5.00^{bB*}	6.66^{aB}	7.00^{aB}	6.55^{aC}	7.00^{aB}
8		5.55^{aC*}	6.67^{aB}	6.00^{aC*}	6.67^{aB}
12			5.67^{bB*}		6.67^{aB}
16					5.00^{C*}
Juiciness					
Zero	8.00^{aA}	8.55^{aA}	8.67^{aA}	8.67^{aA}	8.67^{aA}
4	7.55^{aA}	7.67^{aA}	8.00^{aA}	7.55^{aB}	8.00^{aA}
6	5.00^{bB*}	6.66^{aB}	7.00^{aB}	6.55^{aC}	7.00^{aB}
8		5.55^{bC*}	6.67^{aB}	6.00^{aC*}	6.67^{aB}
12			5.67^{bB*}		6.67^{aB}
16					5.00^{C*}
Overall acceptability					
Zero	8.67^{aA}	8.67^{aA}	9.67^{aA}	8.67^{aA}	9.67^{aA}
4	6.67^{cB}	7.67^{bB}	8.33^{aA}	7.55^{bB}	9.00^{aA}
6	5.00^{dC*}	6.66^{cC}	7.55^{bB}	6.55^{bC}	8.55^{aB}
8		5.55^{cD*}	6.67^{bC}	5.50^{cD*}	8.00^{aB}
12			5.67^{bC*}		7.55^{aC}
16					6.00^{D*}

*Onset of spoilage, Different superscript small characters mean significant differences between different treatments in the same storage periods ($p \leq 0.05$), Different superscript capital characters mean significant differences between different storage periods in the same treatment ($p \leq 0.01$)

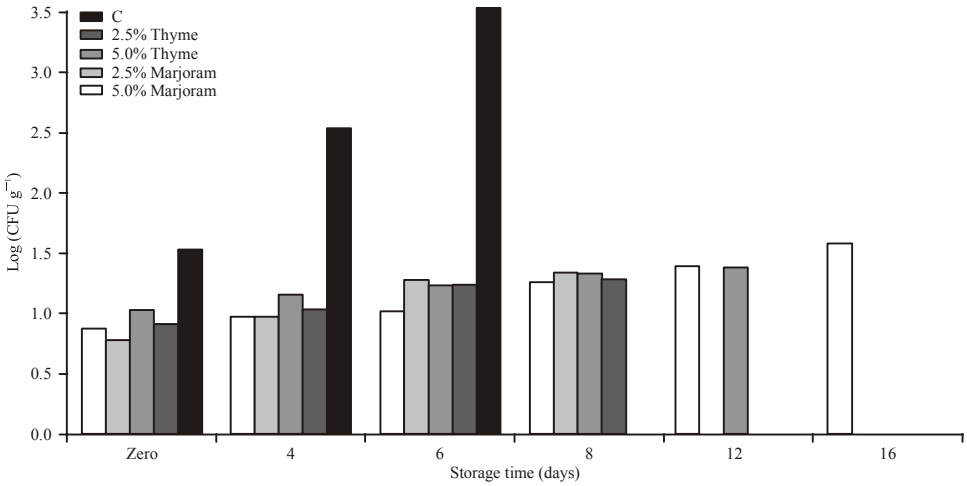

Fig. 3: Enterobacteriaceae counts (CFU g^{-1}) of coated semi fried mullet fish samples during cold storage

fish fillets during cold storage. Results showed that there were lower scores in semi fried mullet fish which did not contain any kinds of spices in its coat (control) than other investigated treatments. There was a significant ($p<0.01$) enhancement in sensory attributes which appeared in samples containing marjoram and thyme at concentrations 2.5 and 5%, compared to control sample. Generally, samples contained 5% marjoram followed by samples contained 5% thyme demonstrated the highest scores than the other treatments.

DISCUSSION

The chemical composition revealed that semi fried process increased the moisture and protein losses by 37.06% and 14.58%, respectively. This loss in moisture content of semi fried fillets was due to evaporation of moisture and elution of components into fried oil. On the other trend, there were incremental pattern in fat, ash and free nitrogen extract. This incremental pattern in the aforementioned parameters may be due to the compounds of coated layer which contained polysaccharide (xanthan) which prevented the loss of fats [14]. In the same time the increased ash content may be due to the spices used in coated layer. Concerning the loss of protein, it may be attributed to decomposition of some protein molecules to volatile nitrogen and further rise of temperature that leads to protein hydrolysis as described by [15]

In this study, this increase of TVB-N in all treatments during cold storage might be attributed to the breakdown protein as a result of activity of microbial strains and proteolysis enzymes [16]. The highest rate of increase was recorded in control fish fillets, e.g., TVB-N reached at the onset of spoilage (after 6 days) 21.00 mg per 100 g wet sample, while it was 18.20, 16.75, 17.50 and 16.60 mg per 100 g wet weight for samples of fish fillets coated 2.5% thyme, 5% thyme, 2.5% marjoram and 5% marjoram, respectively. The treatment with 5% thyme and marjoram was more effective in delaying the formation rate of TVB-N during subsequent cold storage. This is due to the role of such spices on microbial population and the growth of bacteria as antimicrobial agent [17].

From the TMA results, the marjoram has higher effect on the bacterial growth in fish samples during cold storage than thyme in 5%. This is coincided with those of [9]. On the other side, both marjoram and thyme showed the same effect at 2.5% concentration in fish fillets samples during storage. The application of natural antioxidants (thyme and marjoram) in coating layer of mullet fish fillets stored under cold conditions resulted in a decreased microbial populations compared to control samples as proved by several other studies on the essential oils of natural antioxidants [17-19].

Lipid oxidation is one of the main limiting factors for the quality and acceptability of fish and fish products. The use of antioxidants was very effective in reducing lipid oxidation in fish fillets because of its phenolic compounds contents which act as inhibitors for radical reactions on autoxidation [20]. Thus, it has been reported that marjoram and thyme oils may act as scavengers of radicals involved in lipid oxidation protecting lipids from oxidation during frozen and refrigerated storage as discussed by [9, 21, 22]. The initial records of peroxide values of fish samples at the beginning of cold storage were high because of the nature of fish flesh that contained high amount of unsaturated fatty acids which

are easily oxidized and produced high amount of peroxides [16].

From the microbial analysis, one may report that both thyme and marjoram have antimicrobial properties [9]. Marjoram and thyme oils are rich in phenolic compounds being particularly active in both antioxidants and antimicrobials [18]. These results are in accordance with findings of [22, 23], who pointed out that the antimicrobial effect of marjoram and thyme oils constituents were found to be phenols, alcohols, aldehydes, ketones, ethers and hydrocarbons. Specific functional groups introduced into a compound can increase that antimicrobial activity, e.g., phenolic compounds can be bactericidal or bacteriostatic depending on their effective concentration.

There was a general enhancement in all sensory attributes which appeared in samples containing marjoram and thyme at concentrations 2.5 and 5% [24]. This is due to the action of these spices in retarding the oxidation in color and odor as well as in microbial population.

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