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Efficacy of Essential Oils (Thyme and Laurel) on Maintaining the Quality Indices and Nutritional Value of the Frozen Tilapia (*Oreochromis niloticus*) Fillets

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ABSTRACT

The effectiveness of essential oils (thyme and laurel) (EOs, 1%) on the quality indices and nutritional value of tilapia (*Oreochromis niloticus*) fillets stored at -18ºC for 6 months was investigated. Total volatile basic nitrogen (TVB-N), trimethylamine nitrogen (TMA-N), thiobarbituric acid (TBA), pH, amino acids, and total bacterial count (TBC) analyses were carried out every two months. During the frozen storage of fish samples (6 months), the values of TVB-N, TMA-N, TBA, pH and TBC recorded a gradual increase. Until the end of the storage, these values were lower in samples treated with Eos, compared to those in the control, and they were less than the permissible limits. Compared to the control and until the end of the storage period, high nutritional quality was detected in the frozen fish fillets treated with Eos, especially those treated with thyme, followed by laurel and associated with a high value of total amino acids (TAAs), total essential amino acids (TEAAs), amino acid index (AAI) and biological value (BV%). Thus, the potential of EOs to inhibit the biochemical changes in fish samples during frozen storage and maintain their quality was remarkable. Therefore, some essential oils (EOs) are recommended to be used, especially thyme followed by laurel, as antioxidant and antimicrobial agents to improve fish fillets' quality during a long-term freezing process.

INTRODUCTION

Fishes are easily digestible protein, containing considerable amounts of essential amino acids, unsaturated fatty acids, especially omega-3 fatty acids, and form a good source of several minerals particularly fluorine and iodine (**Gulyavuz & Unlusayın, 1999; Gomma, 2005)**. Tilapia is one of the most important farmed species all over the world. Farming of fast growing monosex populations of tilapia produced by hormonal sex reversal of male tilapia, *Oreochromis niloticus* is getting popularized since male tilapia grows nearly twice as fast as females, and its commercial production is increasing worldwide. The most important producers of tilapia today are China, Egypt, Indonesia and the Philippines **(Laly *et al*., 2017)**.

However, fish and other seafood are extremely perishable food products and are especially susceptible to both chemical and microbiological spoilage during processing and/or storage. For this reason, one or more adequate preservation and processing techniques, such as refrigeration, freezing, canning, smoking, salting, drying and also using the essential oils (EOs) as a potential natural antimicrobial and antioxidant agents are required in order to maintain the safety and quality and extend the shelf life of fish and its products **(Ghanbari *et al.*, 2013; Nwachukwu & Madubuko, 2013; Calo *et al.*., 2015; Hassoun & Karoui, 2017)**. Freezing has been considered the most popular method of conservation and successfully employed to maintan the quality of food products, especially fish and other seafood, over long storage periods **(Hall, 2011; Jessen *et al.*, 2014)**. Proper frozen storage minimizes the decline and enhances fish quality **(Solomon & Oluchi, 2018)**. Essential oils (EOs) are produced from different parts of plants, such as leaves, barks, stems, roots, flowers, and fruits as defense mechanisms against microorganisms. These naturally occurring antimicrobial and antioxidant agents are highly complex mixtures of often hundreds of individual aromatic volatile oily compounds **(Jayasena & Jo, 2014; Calo *et al.*., 2015)**. EOs extracted from thyme *(Thymus vulgaris)* and laurel leaves (*Laurus nobilis*) have received much attention from researchers and food processors as potential natural antimicrobial and antioxidant agents due to their high content of phenolic compounds **(Kostaki *et al.*, 2009; Hyldgaard *et al.*, 2012**). Therefore, the present study investigated the effect of commercial essential oils, thyme *(Thymus vulgaris) and* laurel (*Laurus nobilis*), on keeping the quality of the frozen the Nile Tilapia (*Oreochromis niloticus*) fillets at -18ºC for 6 months.

MATERIALS AND METHODS

1. Materials

1.1. Fish:

About 20 kg of the fresh Nile tilapia (*Oreochromis niloticus*) samples were obtained from a fish farm at Shakshouk Research Station for Aquatic Resources (El-Fayoum Governorate), the National Institute of Oceanography and Fisheries (NIOF), Egypt during December 2021. Averages of weight and length were 450±50gm and 27 ±0.50 cm, respectively. Fish samples were carefully washed with tap water and immediately transferred in an ice box to the Fish Processing Technology Laboratory at the same research station**.**

1.2. Ingredients:

Commercial essential oil (EOs), thyme *(Thymus vulgaris)* and laurel (*Laurus nobilis*), were obtained from El-hawag factory, Badr city, Cairo, Egypt. Foam dishes, polyethylene bags were obtained from local market in El-Fayoum city.

2. Methods

2.1. Treatments

Fish samples were beheaded, gutted, eviscerated and the edible part was obtained manually by fish splitting along the side into two fillets. Backbone and skins were

removed, rewashed gently with tap water and drained. Fish fillets samples were divided into 3 equal groups which were dipped individually for 10 minutes at an ambient temperature in the distilled water (control), thyme solution EO (1.0%), and laurel solution EO (1.0%). The treated fillets were packed in polyethylene bags, each of which contained two fillets. All fish fillets were frozen at -18ºC up to 6 months. All parameters for analyses were determined at zero time, 2, 4 and 6 months of frozen storage by taking a randomly sample and thawed overnight at 4± 1ºC. Each analysis was performed in triplicate.

2.2. Analytical methods

Quality Criteria:

Total volatile basic nitrogen (TVB-N) was determined by macro distillation method proposed by **Pearson (1991)**. Trimethylamine nitrogen (TMA-N) was determined according to **A.O.A.C (2012)**. Thiobarbituric acid (TBA, mg MA/kg) value was measured according to the method described by **Pearson (1991)**. The pH value was determined according to **A.O.A.C (2012)**.

Amino Acids:

Amino acids were determined using HPLC & Amino acid analyzer LC3000 Eppendr of Germany in National Research Center according to the method described by **Andrews and Blader (1985)**. The evaluation of nutritional protein was assessed using the determined essential amino acids index (EAAI) calculated according to **Hidvegi and Bekes (1983)**. Biological value (B.V. %) was calculated following the steps of **Oser (1959)**.

Total bacterial count (TBC) was determined using nutrient agar medium according to **Oxoid (1979)**, and the results were expressed as Log_{10}cfu/g sample.

Statistical analyses of the data were performed using the least significant difference test (L.S.D) at $P \leq 0.05$ and standard deviation (Mean ± SD) which were calculated using SPSS 10.0 for windows **(SPSS, 1998)**.

RESULTS AND DISCUSSION

1. Effect of frozen storage at -18ºC for 6 months on total volatile basic nitrogen (TVB-N) of tilapia fillets treated with some commercial essential oils

TVBN is a group of nitrogen-containing compounds, including NH_3 and amines, originated from protein degraded by bacteria and enzymes' activities, and it is considered a marker of the quality and freshness of fish flesh **(Aksnes & Brekken, 1988; Rathod & Pagarkar, 2013)**. The obtained data showing the effect of frozen storage at -18ºC for 6 months on the TVB-N content of the tilapia fillets treated with some commercial essential oils are presented in Table (1) and Fig. (1). TVB-N recorded showed vales of 13.66, 11.05 and 12.40 mg/100g on wet basis (ww) for the control and samples treated by thyme and laurel EOs, respectively, at zero time, and then values increased to 27.18, 22.15 and 24.08 mg/100g after 6 months of the frozen storage. The highest significant ($P<0.05$) incremental of TVB-N values was recorded in the control samples, followed by

samples treated by laurel and treated by thyme, respectively. The TVB-N values of the control and samples treated by EOs gradually increased significantly (*P<0.05*) during the frozen storage period. TVB-N content of the control sample was higher than that of samples treated by thyme and laurel EOs at zero time until the end of storage period due to the effectiveness of EOs as antioxidant and antimicrobial in delaying TVB-N increase. An increase in TVBN content may be attributed to the breakdown of proteins as a result of activity of microbial strains and proteolytic enzymes **(Yassin & Abo-Taleb, 2007)** or may be the result of deamination of free amino acids, oxidation of amines, and degradation of nucleotides by autolytic enzymes and microbial activity **(Ocaño-Higuera *et al.*, 2011).** Therefore, thyme followed by laurel EO are highly effective in delaying the rate of TVB-N increase, as well as in prolonging the shelf-life. This may be attributed to the role of such oils on microbial population and bacterial growth as antimicrobial agents **(Sacchetti *et al.,* 2005)**. In addition, at the end of the frozen storage period (6 months), TVB-N values of the control and treated by laurel and thyme EO did not reach or exceed the acceptability limit (35mg/100 g) stipulated and mentioned by **Lang (1983)**. These results are in harmony with those of **Ibrahim and El-Sherif (2008)** and **Mahmoud *et al*, (2021)**. According to the European Union standards, fish is considered spoiled and unfit for human consumption if it exceeds 25-35mg N/100g for TVN in fish muscles depending on the species of fish **(Ninan *et al*. 2010)**.

Table 1. Effect of frozen storage at -18ºC for 6 months on TVB-N (mg /100g sample w.w.) content of tilapia fillets treated with some commercial essential oils

Storage period (months)	Tilapia fillets			L.S.D at 5%
	Control	Treated by thyme EO	Treated by laurel EO	
0	13.66 ± 0.12	11.05± 0.09	12.40 ± 0.11	1.10
2	15.46 ± 0.11	13.55 ± 0.04	13.90 ± 0.05	0.069
4	19.90 ± 0.08	17.80 ± 0.11	18.65 ± 0.12	0.350
6	27.18 ± 0.13	22.15 ± 0.02	24.08 ± 0.05	0.965
L.S.D at 5%	1.11	1.00	1.02	

Data are calculated as mean ± (SD) standard deviation; n=3, L.S.D 5%: Least significant difference at $P < 0.05$, EOs: Essential oils, TVB-N: Total volatile basic nitrogen, W.W.: On wet weight basis.

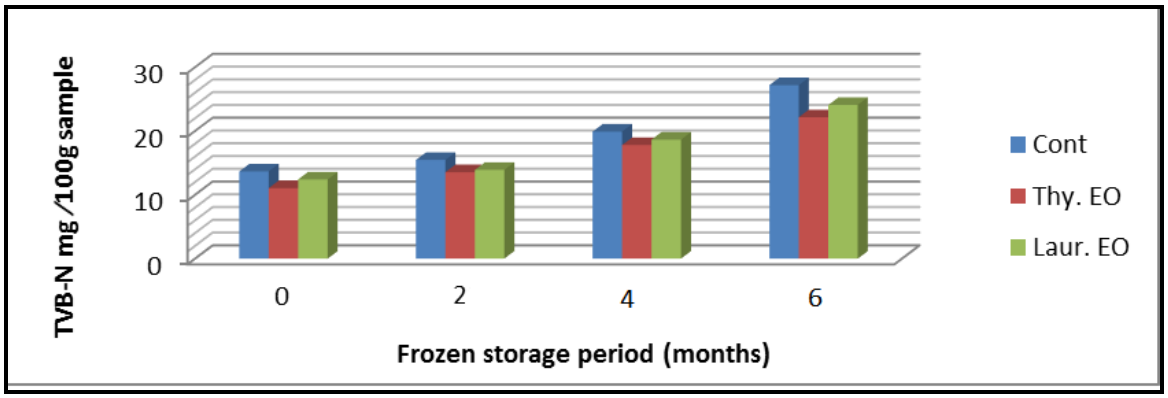

Fig. 1. Effect of frozen storage at -18ºC for 6 months on TVB-N content of tilapia fillets treated with some commercial essential oils.

2. Effect of frozen storage at -18ºC for 6 months on trimethylamine nitrogen (TMA-N) content of tilapia fillets treated with some commercial essential oils.

TMA is produced by the decomposition of TMAO caused by bacterial spoilage and enzymatic activity. Table (2) and Fig. (2) show the effect of thyme and laurel EOs on TMA-N of tilapia fillets during frozen storage at -18ºC for 6 months. Initial average values of TMA-N were 0.78, 0.44 and 0.64 mg/100g on wet basis (ww) for the control and samples treated by thyme and laurel EOs, respectively, at zero time and increased to 1.62, 1.11and 1.11 mg/100g after 6 months of frozen storage. The TMA-N was significantly reduced by treatment with thyme and laurel Eos, compared to the control just after treatment. The highest significant ($P<0.05$) incremental of TVB-N values was recorded in the control sample, followed by samples treated by laurel and thyme, respectively. Values of TMA-N showed statistically significant ($P<0.05$) differences between all treatments at the end of storage period. Therefore, treatments by thyme followed by laurel oils were more effective in delaying the rate of increasing TMA-N as antimicrobial factors compared to the control of tilapia fillets during the frozen storage. Such increment in TMA-N during the frozen storage may be due to the conversion of TMAO to TMA by non enzymatic process**,** or by native tissue enzymes or by bacterial enzymes (TMA ase), which are not completely inactivated by low temperature **(El-Sherif *et al.*, 2011)**. Finally, the tilapia fillets maintained a good quality until the end of frozen storage period; TMA-N content was less than 10-15 mg TMA-N /100g and regarded within the acceptable limits as reported by in the study of **Connell (1990)** as affected by frozen storage process also, antioxidant and antimicrobial agents of thyme and laurel EOs. These results are similar with those of **Abo-Zeid (2020)** and **Abou-Taleb *et al.* (2022)**.

Table 2. Effect of frozen storage at -18ºC for 6 months on trimethylamine nitrogen TMA-N (mg /100g sample w.w.) of tilapia fillets treated with some commercial essential oils

Storage period (months)	Tilapia fillets			L.S.D at 5%
	Control	Thyme EO	Laurel EO	
0	0.78 ± 0.04	0.44 ± 0.13	0.64 ± 0.10	0.008
2	0.91 ± 0.10	0.68 ± 0.10	0.73 ± 0.11	0.011
4	1.05 ± 0.11	0.81 ± 0.10	0.92 ± 0.11	0.005
6	1.62 ± 0.04	1.11± 0.04	1.30 ± 0.10	0.021
L.S.D at 5%	0.102	0.018	0.004	

Data are calculated as mean ± (SD) Standard deviation; n=3, L.S.D 5%: Least significant difference at $P < 0.05$, EOs: Essential oils, TMA-N: Trimethylamine nitrogen, W.W.: On wet weight basis.

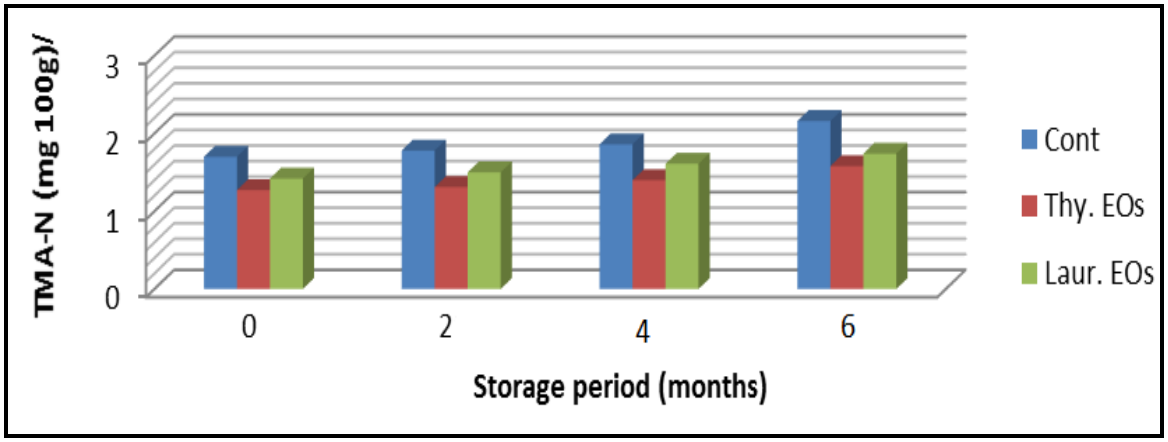

Fig. 2. Effect of frozen storage at -18ºC for 6 months on TMA-N content of tilapia fillets treated with some commercial essential oils

3. Effect of frozen storage at -18ºC for 6 months on thiobarbituric acid (TBA) content of tilapia fillets treated with some commercial essential oils

Lipid peroxidation, corresponding to the oxidative deterioration of polyunsaturated fatty acids in fish muscles leads to the production of off-flavors and off- odors, thereby shortening the shelf-life of food **(Ramanathan & Das, 1992)**. Changes in the TBA value of the tilapia fillets during frozen storage at -18ºC are shown in Table (3) and Fig. (3). Immediately after treatment, there were no significant differences ($P<0.05$) between control and other treated samples. TBA values of control and other treated samples by thyme and laurel EOs were 0.58, 0.22 and 0.31mg (Malonaldhyde) MDA/kg, respectively, at zero time of storage and increased to 1.46, 0.70 and 0.98 mg MDA/kg sample at the end of storage period. A significant ($P<0.05$) increase in TBA of control and other treated samples was observed with the duration of storage and at the end of the storage period that indicated lipid was oxidized during the frozen storage. The

incremental in TBA values may be due to the auto-oxidation of fats and the formation of some TBA-reaction substances during storage, bacteriological and/or oxidative rancidity or may be due to ice crystals formed that could injure the cell, causing the release of pro-oxidants, especially free iron **(Benjakul & Bauer, 2001; Osman & Zidan, 2014; Domínguez *et al.*, 2019)**. A significant ($P \leq 0.05$) increase in TBA value was observed for the control sample, compared to samples treated with thyme and laurel EOs due to their antioxidant effects. This means that the usage of thyme followed by laurel EOs had a positive influence on reducing lipid oxidation. However, as a result of the effect of the the freezing process and Eos, the TBA values of frozen tilapia fillets were less than the permissible limit until the end of the storage period as reported in the study of **Bonnell (1994)** who postulated that, the high quality fish fillets have a TBA value of less than 2.0 mg MDA/kg meat, while lower quality fish contain 3-27 mg MDA/kg. On the other hand, **EOS (2005)** confirmed that, the TBA value in frozen fish should not exceed 4.5mg of malonaldhyde/kg for a sample. These results coincide with those of **Abou-Taleb *et al.* (2007)** and **Karami *et al.* (2013)**.

Table 3. Effect of frozen storage at -18ºC for 6 months on TBA value (mg MDA/kg sample, w.w.) of tilapia fillets treated with some commercial essential oils

Storage period (Months)	Tilapia fillets			L.S.D at 5%
	Control	Thyme EO	Laurel EO	
0	0.58 ± 011	0.22 ± 0.12	0.31 ± 0.18	0.380
2	0.70 ± 0.10	0.38 ± 0.20	0.50 ± 0.09	0.054
4	1.11 ± 0 .13	0.55 ± 0 .12	0.78 ± 0 .21	0.158
6	1.46 ± 0.09	0.70 ± 0.11	0.98 ± 0.05	0.295
L.S.D at 5%	0.109	0.078	0.181	

Data are calculated as mean ± (SD) Standard deviation; n=3, L.S.D 5%: Least significant difference at $P < 0.05$, EOs: Essential oils, TBA: Thiobarbituric acid, W.W.: On wet weight basis.

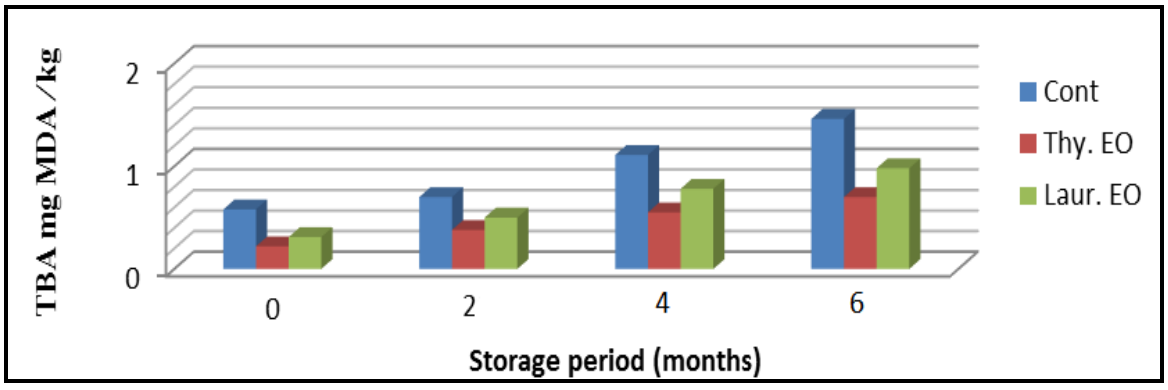

Fig. 3. Effect of frozen storage at -18ºC for 6 months on TBA value (mg MDA/kg sample) of tilapia fillets treated with some commercial essential oils

4. Effect of frozen storage at -18ºC for 6 months on pH value of tilapia fillets treated with some commercial essential oils

Effect of frozen storage at -18ºC for 6 months on pH value of tilapia fillets treated with some commercial essential oils is shown in Table (4) and Fig, (4). pH values were 6.58, 6.42 and 6.42 for the control and samples treated by thyme and laurel EOs, respectively, at zero time of frozen storage, and they increased to 6.82, 6.66 and 6.73 at the end of storage period. No significant difference ($P<0.05$) of pH value was found between the control and other samples treated with thyme and laurel EOs at the beginning of frozen storage. pH values of all the frozen samples were significantly gradually increased ($P<0.05$) with storage time. The recorded increase is due to the presence of alkaline compounds resulted from protein decomposition through autolytic activities or microbial metabolism **(Liu *et al.*, 2010; Lago *et al.*, 2019).** pH values were significantly ($P<0.05$) higher in control samples than those treated with Eos. This may be due to the effectiveness of these EOs as an antimicrobial agents with inhibitory effects on microbial growth, which in turn, delays the formation of basic nitrogen compounds; pH value was higher in samples treated with laurel than treated with thyme. This confirmed that thyme followed by laurel EOs as antimicrobial agents were more effective in delaying the increase of pH value during the frozen storage, compared to the control. These results are similar to those reported in the studies of **Ibrahim and El-Sherif (2008)**, **El-Lahamy *et al.* (2018)** and **Mohamed *et al.* (2019)**.

Table 4. Effect of frozen storage at -18ºC for 6 months on pH value of tilapia fillets treated with some commercial essential oils

Storage period (Months)	Tilapia fillets			L.S.D at 5%
	Control	Thyme EO	Laurel EO	
0	6.58 ± 0.10	6.42 ± 0.10	6.42 ± 0.08	0.002
2	6.62 ± 0.11	6.48 ± 0.09	6.52 ± 0.11	0.004
4	6.78 ± 0.04	6.55 ± 0.10	6.61 ± 0.20	0.010
6	6.82 ± 0.06	6.66 ± 0.06	6.73 ± 0.09	0.015
L.S.D at 5%	0.005	0.003	0.001	

Data are calculated as mean ± (SD) Standard deviation; n=3, L.S.D 5%: Least significant difference at $P < 0.05$, EOs: Essential oils, W.W.: On wet weight basis.

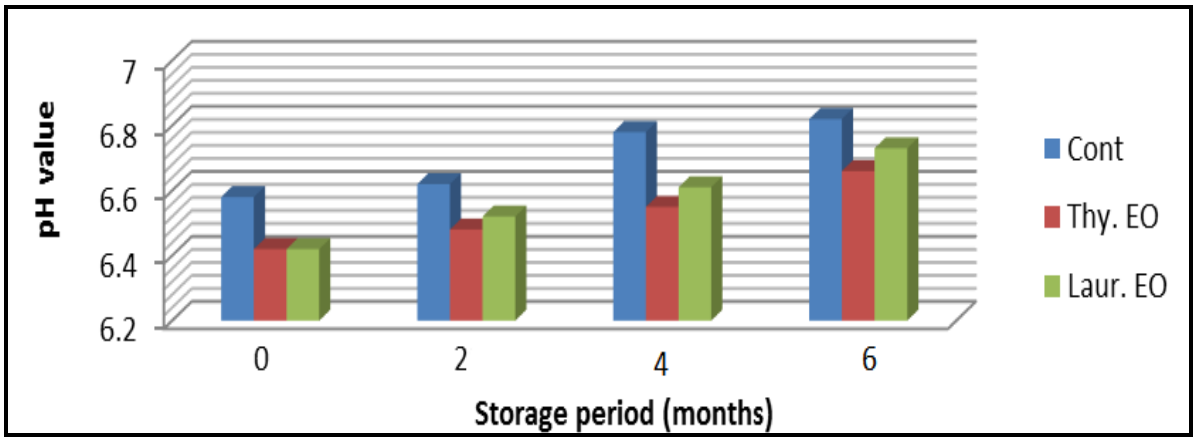

Fig. 4. Effect of frozen storage at -18ºC for 6 months on pH value of tilapia fillets treated with some commercial essential oils

5. Effect of frozen storage at -18ºC for 6 months on amino acid composition and protein quality of tilapia fillets treated with some commercial essential oils

The effect of frozen storage at -18ºC for 6 months on amino acid composition and protein quality of tilapia fillets treated with some commercial essential oils is presented in Table (5). In this study, seventeen different amino acids were detected in tilapia fillets; 10 essential amino acids and 7 nonessential amino acids. Tilapia fillets are rich in essential amino acids (EAA) particularly lysine, leucine and isoleucine, while the high values of non-essential amino acids (NEAA) were found in glutamic and aspartic acids. From the obtained data, most of the amino acids were reduced, while the other increased as affected by freezing. EAA; lysine recorded values of 7.40, 7.68, 7.22 g/16gN in the control and samples treated with thyme and laurel EOs, respectively, at zero time of frozen storage and decreased to 7.05, 7.55, 7.10 g/16gN after 6 months of frozen storage (end storage). Leucine values were 5.85, 6.05 and 5.97 g/16gN in the control and samples treated with thyme and laurel EOs, respectively, at zero time of frozen storage, and decreased to 5.12, 6.00 and 5.31 g/16gN at the end of storage. The values of sulphur containing amino acids (cystine and methionine) were 3.21, 3.72 and 3.50 g/16gN in the control and samples treated with thyme and laurel EOs, respectively, at zero time of frozen storage, and values decreased to 2.85, 3.54 and 3.51 g/16gN at the end of the storage period. NEAA; glutamic acid recorded values of 11.80, 12.02, 11.50 g/16gN in the control and samples treated with thyme and laurel EOs, respectively, at zero time of frozen storage and decreased to 11.06, 11.90, 11.08 g/16gN at end storage. Aspartic acid recorded values of 9.10, 9.15 and 9.00 g/16gN in the control and samples treated with thyme and laurel EOs, respectively, at zero time of frozen storage and decreased to 8.76, 3.54 and 3.51 g/16gN at the end of storage. Proline recorded values of 6.72, 6.80 and 6.55 g/16gN in the control and samples treated with thyme and laurel EOs, respectively,

at zero time of frozen storage and increased to 7.15, 6.90 and 6.83 g/16gN at the end of storage. The observed increase and decrease in some amino acids in tilapia fillets during the frozen storage could be due to the conversion of some amino acids to others during the processes of oxidation or deamination, or the damage of some amino acids during the freezing and thawing process **(Rahimzade *et al.*, 2019)**. The high nutritional quality was confirmed of tilapia fillets as indicated by high of total amino acids (TAA), total essential amino acids (TEAA), TEAA / TNEAA, essential amino acids index (EAAI), and biological value (B.V.%); however, the changes of the nutritional quality during frozen storage for control and treated samples with thyme and laurel EOs were noticed. Values of TAA, TEAA, EAAI and B.V.% were 83.14, 38.59, 75.94 g/16gN, and 71.04%, respectively, in the control at zero time of frozen storage and decreased to 79.77, 35.23, 69.41 g/16gN and 63.93%, respectively, at the end of frozen storage. They were 86.47, 40.91, 81.45 g/16gN and 77.05%, respectively, in samples treated with thyme at zero time of frozen storage and decreased to 84.95, 39.25, 76.63 g/16gN and 77.05%, respectively at the end of frozen storage. While, they were 84.13, 40.15, 79.44 g/16gN and 74.86%, respectively in samples treated with laurel EO at zero time of frozen storage, and they decreased to reach 84.95, 39.25, 76.63 g/16gN and 77.05%, respectively, at the end of frozen storage. This decrease in the nutritional quality (TAA, TEAA, EAAI, B.V. %) during the frozen storage is due to the **Table (6).** Effect of frozen storage at -18ºC for 6 months on total bacterial count (TBC) of Tilapia fillets treated with some commercial essential oils.effect of the freezing process, and may be attributed to the breakdown of proteins as a result of the activity of microbial strains and proteolytic enzymes **(Yassin & Abo-Taleb, 2007)**.

From these data, it could be concluded that samples treated with thyme EO were higher with respect to the nutritional quality (TAA, TEAA, EAAI, B.V. %), followed by samples treated with laurel EO, compared to control samples. This is due to the effectiveness of these EOs since the antimicrobial agents show inhibitory effects on the microbial growth. This finding concurs with that of **El-Lahamy (2018)** and **Rahimzade *et al.* (2019)**.

Table 5. Effect of frozen storage at -18ºC for 6 months on amino acid composition and protein quality of tilapia fillets treated with some commercial essential oils

Amino acids	Control		Thyme EO		Laurel EO	
	g/16g N 0 time	g/16g N 6 month	g/16g N 0 time	g/16g N 6 month	g/16g N 0 time	g/16g N 6 month
Aliphatic:						
Isoleucine (Ile.)*	5.22	4.82	5.58	5.42	5.45	5.22
Leucine (Leu.)*	5.85	5.12	6.05	6.00	5.97	5.31
Threonine (Thr.)*	3.00	2.86	3.36	3.22	4.05	3.00
Valine (Val.)*	4.95	4.25	4.86	4.80	4.90	4.76
Serine (Ser.)	4.15	4.32	4.40	4.33	4.00	4.22
Glycine (Gly.)	3.25	3.25	3.50	3.62	3.32	3.30
Alanine (Ala.)	4.65	4.88	4.71	4.80	4.65	4.72
Aromatic:						
Phenylalanine (Phe.)*	3.86	3.40	4.05	3.55	3.91	3.46
Tyrosine (Tyr.)*	2.05	2.00	2.33	2.25	2.10	2.10
Phe. + Tyr.	5.91	5.40	6.38	5.70	6.01	5.56
Sulphur:						
Methionine (Met.)*	2.55	2.20	3.07	2.86	2.85	2.71
Cystine (Cys.)*	0.66	0.65	0.65	0.68	0.65	0.80
Met. + Cys.®	3.21	2.85	3.72	3.54	3.50	3.51
Heterocyclic:						
Tryptophan (Try.)*	ND	ND	ND	ND	ND	ND
Proline (Pro.)	6.72	7.15	6.80	6.90	6.55	6.83
Acidic:						
Aspartic acid (Asp.)	9.10	8.76	9.33	9.15	9.25	9.00
Glutamic acid (Glu.)	11.80	11.06	12.02	11.90	11.50	11.08
Basic:						
Histidine (His.)*	3.05	2.88	3.28	3.02	3.05	2.88
Lysine (Lys.)*	7.40	7.05	7.68	7.55	7.22	7.10
Arginine (Arg.)	4.88	5.12	4.80	5.00	4.71	4.92
TAA	83.14	79.77	86.47	84.95	84.13	81.41
TEAA	38.59	35.23	40.91	39.25	40.15	37.34
TNEAA	44.55	44.54	45.56	45.70	43.98	44.07
TEAA/TNEAA	0.87	0.79	0.90	0.86	0.91	0.85
EAAI	75.94	69.41	81.45	76.63	79.44	75.56
B.V. (%)	71.04	63.93	77.05	71.70	74.86	70.63

*: Essential amino acids (EAA), TAA: Total amino acids. TEAA: Total essential amino acids, TNEAA: Total nonessential amino acids, EAAI: Essential amino acid index, BV%: Biological value. ND: Not determined.

6. Effect of frozen storage at -18ºC for 6 months on total bacterial count (TBC) of tilapia fillets treated with some commercial essential oils

Table (6) and Fig. (5) explain the effect of frozen storage at -18ºC for 6 months on total bacterial count (TBC) of tilapia fillets treated with some commercial essential oils. At zero time of frozen storage, the control samples showed the highest mean counts of TBC, compared to samples treated with thyme and laurel Eos.A significant ($P<0.05$) reduction was detected in the microbial count of treated fish fillets with EOs immediately after preparation of the antimicrobial agents. This antimicrobial activity of these essential oils could be due to the hydrophobic nature that enables them to split lipids in the bacterial cell membrane and mitochondria, thus breaking down the structures and making them more permeable (**Sikkema *et al.*, 1994**). From data, TBC reduced in control fillets and EOs-treated fillets until the end of the second month, and then gradually increased ($P<0.05$) during frozen storage until the end of the storage period (6 months) with a significant increase in TBC for control fillets followed by of fillets treated by laurel and then fillets treated by thyme EOs. The values of initial TBC (log_{10}cfu/g sample) for control and samples treated by thyme and laurel EOs were 4.10, 3.40 and 3.80 at zero time of storage and decreased to 3.88, 3.25 and 3.66, respectively after two months **(Ibrahim and El-Sherif, 2008 ; Obemeata *et al.* (2011)** and significantly ($P<0.05$) gradually increased to 4.65, 3.85 and 4.05 at the end of the storage period (**EL-Sherif *et al.*, 2011).** The reduction in the microbial load could be explained initially due to the freezing and the powerful antimicrobial properties EOs additives. While, the increase of TBC during frozen storage until the end of storage period may be attributed to the increase in simple nitrogenous compounds (amino acids, nucleotides and free fatty acids) which produced by hydrolysis of protein and fat by natural fish enzymes which consequently lead to suitable conditions for bacterial growth.

However, these levels of TBC at the end of frozen storage period very little and did not exceed the maximum limits (7 log cfu/g) as set for fresh and frozen fish products given by the International Commission on Microbiological Specifications for Foods **(ICMSF, 2002).** This decrease of TBC may be due to the freezing process and the effect of the used EOs as antimicrobial and antioxidants agents had inhibitory effects on microbial growth. These results are harmony with those reported by **Erkan *et al*. (2011) who** showed that the shelf life of stored bluefish in ice for 13 days has extended by 3–4 days compare to the control samples. Also, the similar results were found by **Vanitha *et al.* (2013) and El-Lahamy (2018)**.

Table (6). Effect of frozen storage at -18ºC for 6 months on total bacterial count (TBC) of Tilapia fillets treated with some commercial essential oils.

Storage period (Months)	Tilapia fillets			L.S.D at 5%
	Control	**Thyme EO**	**Laurel EO**	
0	4.10 ± 0.11	3.40 ± 0.08	3.80 ± 0.06	0.015
2	3.88 ± 0.05	3.25 ± 0.10	3.66 ± 0.05	0.015
4	4.22 ± 0.09	3.52 ± 0.10	3.82 ± 0.11	0.015
6	4.65 ± 0.10	3.85 ± 0.09	4.05 ± 0.10	0.015
L.S.D at 5%	0.012	0.009	0.011	

Data are calculated as mean ± (SD) Standard deviation; n=3, L.S.D 5%: Least significant difference at P < 0.05, EOs: Essential oils, TBC: Total bacterial count, cfu: colony forming units, W.W.: On wet weight basis.

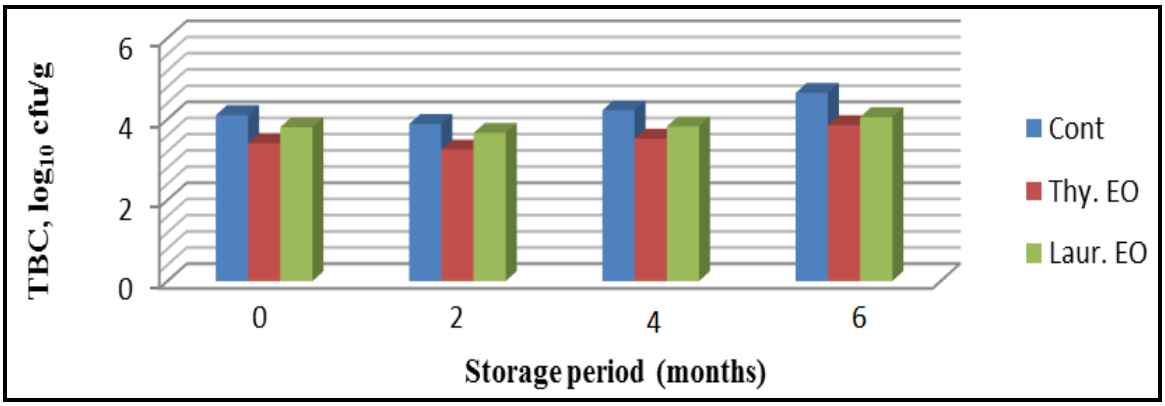

Figure (5). Effect of frozen storage at -18ºC for 6 months on total bacterial count (TBC) of Tilapia fillets treated with some commercial essential oils.

CONCLUSION

In conclusion, this study recommends using of some essential oils (EOs), especially thyme followed by laurel for fish fillets that intended for storage at low temperature, since these EOs have a role in inhibiting the biochemical changes and quality loss, especially thiobarbituric acid value, amino acids and bacterial load. Therefore, the essential oils are available and can be used as antioxidant and antimicrobial agents to improve fish fillets quality during long-term freezing.

REFERENCES

A.O.A.C. (2012). Association of official analytical chemists. Official Methods of Analysis, 19th edition. USA.

Abou-Taleb, M. ; El-Sherif, S. A. and Elhariry, H. (2007). Preservation effect of four plant extracts used to extending the shelf-life of mullet fish fillets during cold storage. World Journal of Dairy and Food Sciences 2 (2): 74-82.

Abou-Taleb, M. ; Talab, A. S. ; Ibrahim, M. A. ; Abo-Zeid, K. S. and Mahmoud, M. M.(2022). Effect of some plant extracts on the quality of fish sausage and burger. Egyptian Journal of Aquatic Biology & Fisheries, 26(1): 287-297.

Abo-Zeid, K. S. (2020). Technological studies on Catfish (*Clarias gariepinus)* from Wadi El-Rayan Lake, Fayoum to produce varied fish products. Ph. D Thesis, Fac. of Agric., Al-Azhar Univ., Egypt.

Aksnes, A. and Brekken, B. (1988). Tissue degradation, amino acid liberation and bacterial decomposition of bulk stored capelin. Journal of the Science of Food and Agriculture, 45(1): 53-60.

Andrews, S. R. and Blader, N. (1985). Amino acid analysis of feed constituents. Science Tools, 32:44.

Benjakul, S. and Bauer, F. (2001). Biochemical and physicochemical changes in catfish (*Silurus glanis Lines*) as influenced by different freeze-thaw cycles. Food Chem., 72:207-217.

Bonnell, A. D. (1994). Quality Assurance in Seafood Processing: A Practical Guide. Chapman and Hall, London, U.K., pp. 74-75.

Calo, J. R. ; Crandall, P. G. ; O'Bryan, C. A. and Ricke, S. C.(2015). Essential oils as antimicrobials in food systems - a review. Food Control (54):111-121.

Connell, J. J. (1990). Control of Fish Quality. Fishing News Book, a Division of Black well Scientific Publication, Cambridge U.K..

Domínguez, R. ; Mirian, P. ; Mohammed, G. ; Francisco, J. B. ; Wangang, Z. and José M. L., A. (2019). Comprehensive Review on Lipid Oxidation in Meat and Meat Products. Antioxidants, 8 (10): 429-460.

El-Lahamy, A. A. (2018). Effect of some processing methods on quality of some fishes of Wady El-Rayan, Fayoum Governorate. Ph. D Thesis, Fac. of Agric., Fayoum Univ., Egypt.

El-Lahamy, A. A. ; Khalil, K. I. ; El-Sherif, S. A. and Mahmud, A. A. (2018). Changes in quality attributes of sand smelt (*Atherina hepsetus*) fish burger and finger during frozen storage. J. Fish. Res., 2 (2): 1-11.

El-Sherif, S. A. ; Ibrahim, S. M. and Abou-Taleb, M. (2011). Relationship between frozen pre-storage period on raw Tilapia and Mullet fish and quality criteria of its cooked products. Egyptian Journal of aquatic Research, 37 (2):183-189.

EOS (2005). Egyptian Organization for Standardization and Quality Control. Maximum residual limit of heavy metals in food. Ministry of Industry, No. 2360/2005. Cairo, Egypt.

Erkan, N. ;Tosun, S. Y. ; Ulusoy, S. and Üretener, G. (2011). The use of thyme and laurel essential oil treatments to extend the shelf life of bluefish (*Pomatomuss altatrix*) during storage in ice. J. Verbr. Lebensm, 6; 39-48.

Ghanbari, M. ; Jami, M. ; Domig, K. J. and Kneifel, W. (2013). Seafood bio preservation by lactic acid bacteria-a review. LWT-Food Science and Technology (54): 315-324.

Gomma, R. A. M. (2005). Studies on producing sausage from some fish types. M.Sc. Thesis, Fac.of Agric. Al-Azher Uni., Egypt.

Gulyavuz, H. and Unlusayın, M. (1999). Fish Processing Technology, ISBN: 975 – 968897 – 0 , Ankara, Turkey.

Hall, G. M. (2011): Freezing and chilling of fish and fish products. In G. M. Hall (Ed.), Fish processing-sustainability and new opportunities. Blackwell Publishing Ltd., 77–97.

Hassoun, A. and Karoui, R. (2017). Quality evaluation of fish and other seafood by traditional and nondestructive instrumental methods: Advantages and limitations. Critical Reviews in Food Science and Nutrition (57): 1976-1998 (2017).

Hidvegi, M. and Bekes, F.(1983). Mathematical modeling of protein nutritional quality from amino acids composition. Proc. Int. Assoc., Cereal Chem. Symp. Amino acid composition and biological value of cereal proteins. Budabest, Hungari.

Hyldgaard, M. ; Mygind, T. and Meyer, R. L. (2012). Essential oils in food preservation: Mode of action, synergies, and interactions with food matrix components. Frontiers in Microbiology, 3:1-24.

Ibrahim, S. M. and El-Sherif, S. A. (2008). Effect of some plant extracts on quality aspects of frozen Tilapia *(Oreachromis niloticus L.)* Global Veterinaria 2 (2): 62-66.

ICMSF (2002),International Commission on Microbiological Specifications for Foods. Microorganisms in Foods: Microbiological Testing in Food Safety Management, Kluwer Academic/Plenum Publishers, New York, 258-259pp.

Jayasena, D. D. and Jo, C. (2014). Potential application of essential oils as natural antioxidants in meat and meat products: A review. Food Reviews International, 30: 71-90.

Jessen, F. ; Nielsen, J. and Larsen, E. (2014). Chilling and freezing of fish. In I. S. Boziaris (Ed.), Seafood processing: Technology, quality and safety pp. 98-118 .

Karami, B. ; Moradi, Y. ; Motallebi, A. A. ; Hosseini, E. and Soltani, M. (2013). Effects of frozen storage on fatty acids profile, chemical quality indices and sensory properties of red Tilapia (*Oreochromis niloticus* × *Tilapia mosambicus*) fillets. Iranian Journal of Fisheries Sciences, 12(2): 378-388.

Kostaki, M. ; Giatrakou V. ; Savvaidis I. N. and Kontominas M. G. (2009). Combined effect of MAP and thyme essential oil on the microbiological, chemical and sensory attributes of organically aquacultured sea bass (*Dicentrarchus labrax*) fillets. Food Microbiology, (26): 475-482.

Lago, A. M. T. ; Teixeira, J. T. ; Olímpio, B. J. G. ; Schiassi, M. C. E. V. ; Pimenta, C.J. and Gomes, M. E. S.(2019). Shelf life determination of frozen fish sausage produced with fillet and minced fish derived from the Nile Tilapia processing. J Food Process Preserv., 43: e 13984.

Laly, S. J., Kumar, K. N., Sankar, T. V., Lalitha, K.V., Ninan, G.(2017). Quality of monosex Tilapia under ice storage: Gutting effects on the formation of biogenic amines, biochemical, and microbiological characteristics. International Journal of Food Properties, 20 (6): 1368-1377.

Lang, K.(1983). Der fluchtige basenstickstoff (TVB-N) bei im binnenland in den verkehr gebrachten frischen seeficchen. 11. Mitteilung. Archiv für Lebensmittelhygiene, 34: 7-10.

Liu, S. ; Fan, W. ;Zhong, S. ; Ma, C. H. ; Li, P. ; Zhou, K. Peng, Z. and Zhu, M. (2010). Quality evaluation of tray-packed Tilapia fillets stored at 0 ºC based on sensory, microbiological, biochemical and physical attributes. African Journal of Biotechnology, 9(5): 692-701.

Mahmoud, Eman A. ; Mohamed, Eman and Sharaf, A. M.(2021). Quality assessment of Egyptian Catfish (*Clarias gariepinus*) fillet during frozen storage. J. of Food and Dairy Sci., Mansoura Univ., 12 (10):253-258.

Mohamed, H. R. ; El-Lahamy, A. A. ; Ibrahim, S. M. ; Hafez, N. E.and Awad, A. M. (2019). Changes in biochemical criteria of Tilapia fish samples during frozen storage at-18ºC for 180 days and their fried products. Journal of Experimental Food Chemistry, 5 (1): 1-5.

Ninan, G. ; Bindu, J. and Joseph, J. (2010). Frozen storage studies of value added mince based products from Tilapia (*Oreochromis mossambicus*, Peters 1852). Journal of Food Processing and Preservation, (34): 255-271.

Nwachukwu, V. N. and Madubuko, C. U. (2013): Microflora associated with processing and storage of the white catfish (*Chrysichthys nigrodigitatus*), Journal of Fisheries and Aquatic Science, 8 (1): 108-114.

Obemeata, O. and Nnenna, F. P.(2011). Christopher, N., Microbiological assessment of stored Tilapia genesis. Africa. J. Food Sci. 5(4):242-247.

Ocaño-Higuera, V. M. (2011). Freshness assessment of ray fish stored in ice by biochemical, chemical and physical methods. Food Chemistry 125 (1): 49-54.

Oser, B. L. (1959) An integrated essential amino acid index predicting the biological value of protein and amino acid nutrition. Ed. A.A. Albannese, Academic press, New York.

Osman, M. F. and Zidan, N. S.(2014). Quality assessment of formulated fish silver carp burgers during frozen storage. J. Food and Dairy Sci., Mansoura Univ., 5 (6): 403-419.

Oxoid, M.(1979). The Oxoid Manual of Culture Media and Other Labortary service. Fourth Edition.

Pearson, D.(1991). the Chemical Analysis of Food. Churchill, New York, London, P: 374-410.

Rahimzade, E.B; Bhri, A. H. ;Moini, S. and Nokhbe Zare, D.(2019). Influence of vacuum packaging and frozen storage time on fatty acids, amino acids and ω -3/ ω-6 ratio of rainbow trout (*Oncorhynchus mykiss*). Iranian Journal of Fisheries Sciences 18(4) 1083-1092.

Ramanathan, L. and Das, N. B. (1992). Studies on the control of lipid oxidation in ground fish by some polyphenolic national products. J. Agric. Food Chem., 40: 17-21.

Rathod, N. and Pagarkar, A. (2013). Biochemical and sensory quality changes of fish cutlets, made from pangasius fish (*Pangasianodon hypophthalmus*), during storage in refrigerated display unit at -15 to -18°C. International Journal of Food Agriculture Vet. Science, 3(1): 1-8.

Sacchetti, G. ;Maietti, S. ; Muzzoli, M. ; Scaglianti, M. ; Manfredini, S. ; Radice, M. and Brni, R.(2005). Comparative evaluation of 11 essential oils of different origin as functional antioxidants, antiradicals and antimicrobials in foods. Food Chem., 91; 621-632.

Sikkema, J. ; DeBont, J. A. M. and Poolman, B.(1994). Interactions of cyclic hydrocrbons with biological membranes. J. Biol. Chem., 269 (11): 8022-8028.

Solomon, R. J. and Oluchi, A. R. (2018). Proximate Analysis and Nutritional Value of African Catfish (*Clarias gariepinus*) Fed with Local (*Telferia occidentales and Moringa olefera*) and Industrial Feed (Coppens). *Journal of Fisheries and Livestock Production* (6): 267.

SPSS (1998). Statistical Package for the Social Science, SPSS 9.0 regression models, Chicago, IL:SPSS.

Vanitha, M. ; Dhanapal, K. ; Sravani, K.and Reddy, **V. S. G., (2013).** Quality evaluation of value added mince based products from catla (*Catla catla*) during frozen storage. International Journal of Science, Environment and Technology, 2 (3): 487-501(2013).

Yassin-Nessrien, M. and Abou-Taleb, M., (2007): Antioxidant and antimicrobial effects of marjoram and thyme in coated refrigerated semi fried mullet fish fillets. World J. Dairy & Food Sci. 2 (1): 1-9.