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Effect of Some Essential Oils on Biogenic Amines in Fishes at Kalyobia Governorate

S.M.El-Khabaz[1],H.M.Ibrahim[2] and R.A.Amin[2]
[1]Director of Veterinary Medicine, Qalyubiya
[2]Food Hygeine Control Department, Faculty of Veterinary Medicine, Benha Univ., Benha, Egypt

Abstract

A grand total of 21 samples of fresh fish fillets (*Oreochromis niloticus*) were collected randomly from different fish markets in Benha, Kalyobia governorate to determine the effect of some essential oils"clove oil(1%), thyme oil (1%) and lemon oil"(1%) at4°C for 2and 4 days on their contents of various biogenic amines "histamine, putrescine, cadaverine and tyramine". The results indicated that histamine levels in the examined fish fillets were reduced by 5.5% - 14.75% by addition of clove oil (1%), 7.25 - 20.0% by addition of thyme oil (1%) and 15.8% - 29.3% by addition of lemon oil (1%) after 2 and 4 days, respectively. While, addition of clove oil (1%), thyme oil (1%) and lemon oil (1%) cause reduction toputrescine by 9.6 - 11.9%, 12.5 - 16.4% and 19.35% - 34.1% after 2 and 4 days, respectively. Concerning to cadaverine levels, the reduction occurred by 7.7 – 9.3%, 10.4 – 22.9% and 15.8% - 29.8% after 2 and 4 days, respectively. Finally, addition of clove oil (1%), thyme oil (1%) and lemon oil (1%) cause reduction totyramine by 5.2 – 10.6%, 16.5 - 16.3% and 22.4% - 25.1% after 2 and 4 days, respectively. The obtained results it allow concluding that the addition of essential oils lead to reduction of biogenic amines in fish fillets eliminating the hazard effect of biogenic amines on human.

Keywords: Fish Fillet, Biogenic Amines, Clove Oil, Thyme Oil, Lemon Oil.

1. Introduction

Fish meat share in solving the shortage in animal protein requirement; it is the most important single source of high-quality protein, providing nearly 16% of the animal protein consumed by the world's population **[1]**.Moreover, fish oil is a good source of calories and provides many important vitamins as B group, A and D, beside calcium, phosphorus and iodine **[2]**. Biogenic amines (BAs) occur in a wide variety of foods including fish and fish products **[3]**. They have an important metabolic role in living cells; some of them are essential for growth and others involved in nervous system functions**[4]**. High amounts of certain amines may be found in food as a consequence of the use of poor quality raw materials, contamination and inappropriate conditions during food processing and storage **[5]**. In this respect, the quantity of biogenic amines is supposed to be a marker for microbiological contamination level in the food **[6]**. Thus, analysis of biogenic amine is important because of their toxicity and they can be used as an indicator for the degree of freshness or food spoilage **[7]**. In fish, many BAs have been found, but only histamine, cadaverine, and putrescine have been identified as significant concerns with fish safety and quality. Histamine alone appears to be insufficient to cause toxicity, but putrescine and cadaverine potentiate its toxic activity **[8]**. Regardless to fish spoilage, only cadaverine has been found to be a useful index of the initial stage of fish decomposition **[9]**.Fresh fish contains little or no tyramine, but a large amount can be found in spoiled or fermented fish**[10]**.

From public health hazard of view,tyramine has a direct effect on increasing blood pressure through contraction of blood vessel **[11]**.Putrescine and cadaverine can react with nitrite to form heterocyclic carcinogenic nitrosamine, nitrosopyrrolidine and nitrosopiperidine**[4]**.

Essential oils and their components are commonly used as flavoring agents in food industry, also they have some antibacterial, antifungal and antioxidant properties used for extending the shelf life and maintaining the quality of food **[12]**.Essential oils contain a wide variety of secondary metabolites that are capable of inhibiting or slowing the growth of bacteria **[13**]. Thyme oil is well known for their antimicrobial activity mainly due to their content of phenolic compounds.Lemon oil is one of the most essential oils. Lemon and lemon essential oil are used to decrease cholesterol deposition in the blood vessels and prevent heart disease.

Therefore, the aim of the current study is to determine the effect of some essential oils"clove oil, thyme oil and lemon oil "by concentrations 1% at 4°C for 2 days and 4 dayson the levels of biogenicamines"histamine, putrescine, cadaverine and tyramine" in the examined fishes fillets.

2. Matreial and methods

A grand total of 21 samples of fish fillets (*Oreochromis niloticus*) were divided into 7 groups (3 samples for each group) as follow:

1. The 1st group represented control one.
2. The 2nd group was immersed in clove oil (1%) and stored in refrigeratorat4°C for2 days.
3. The 3rd group was immersed in clove oil (1%) and stored in refrigerator at4°Cfor 7 days.

4. The 3rd group was immersed in thyme oil (1%) and stored in refrigerator at4°C for 2 days.
5. The 4th group was immersed in thyme oil (1%) and stored in refrigerator at 4°Cfor 4 days.
6. The 3rd group was immersed in lemon oil (1%) and stored in refrigerator at4°C for 2 days.
7. The 4th group was immersed in lemon oil (1%) and stored in refrigerator at 4°C for 4 days.

Accurately, 50 gm of each fish fillet sample were immersed in 100 ml of each tested essential oil for 15 minutes. All samples either control or treated ones were analyzed for determination of their levels of biogenic amines by HPLC.

3. Results and discussion

The results achieved in table (1) declared the effect of essential oils (1%) on histamine levels (mg%) in the examined fish samples. In which the mean value of histamine level in the control group at the zero time is 26.73 ± 1.16, while after 2 days and 4 days (Storage time at 4°C) were 32.28 ± 1.4 and 43.91 ± 2.32, respectively. The addition of clove oil (1%) decreased histamine levels to mean value of 30.52 ± 1.39 and 37.46 ± 1.78 after 2 days and 4 days (Storage time at 4°C), respectively. While by addition of thyme oil (1%) the progression of histamine levels decreased to the mean value of 29.95 ± 1.31 and 35.12 ± 1.48 after 2 days and 4 days (Storage time at 4°C), respectively. Furthermore, the addition of lemon oil (1%) cleared the highest reduction in histamine levels to the mean value of 27.19 ± 1.10 and 31.05 ± 1.22 in 2 days and 4 days (Storage time at 4°C), respectively. The progression of histamine formation was clearly reduced by the percentage of 5.5% -14.7%, 7.2% - 20.0% and 15.8% - 29.3% after addition of clove oil, thyme oil and lemon oil (1%) for 2 days and 4 days (storage time at 4°C).

The results accomplished in Table (2) proclaimed the effect of essential oils (1%) addition on putrescine levels (mg %) in the examined fish samples, from which the mean value of putrescine level in zero time for the control group is 26.73 ± 1.16, while become 32.28 ± 1.40 and 43.91 ± 2.32 after 2 days and 4 days (Storage time at 4°C), respectively.

Furthermore addition of clove oil (1%) decreased the progression level of putrescine to mean value of 30.52 ± 1.39 and 37.46 ± 1.78 after 2 days and 4 days (Storage time at 4°C), respectively. While the addition of thyme oil (1%) decreased the progression level of putrescine to the mean value of 29.95 ± 1.31 and 35.12 ± 1.48 after 2 days and 4 days (Storage time at 4°C), respectively. Finally, the addition of lemon oil (1%) gives the highest reduction in putrescine levels with the mean value of 27.19 ± 1.10 and 31.05 ± 1.22 after 2 days and 4 days (Storage time at 4°C), respectively. The progression of putrescine formation was clearly reduced by the percentage of 9.6% - 11.9%, 12.5% - 16.4% and 19.3% - 34.1% after addition of clove oil, thyme oil and lemon oil (1%) for 2 days and 4 days (storage time at 4°C).

The results recorded in table (3) cleared the addition effect of essential oils (1%) on cadaverin levels (mg %) in the examined fish samples, in which the mean value of cadaverin level in control group at the zero time is 9.57 ± 0.69, and 12.03 ± 0.81 and 16.49 ± 1.05 after 2 days and 4 days (Storage time at 4°C), respectively. The addition of clove oil (1%) decreased the progression of cadaverin levels to mean value of 11.57 ± 0.69 and 14.96 ± 0.98 after 2 days and 4 days (Storage time at 4°C), respectively. While addition of thyme oil (1%) reduced the progression of cadaverin level to mean value 10.78 ± 0.80 and 12.72 ± 1.14 after 2 days and 4 days (Storage time at 4°C), respectively. Finally, the addition of lemon oil (1%) gives the highest reduction in cadaverin levels to the mean value 10.13 ± 0.72 and 11.58 ± 1.01 after 2 days and 4 days (Storage time at 4°C), respectively.The progression of cadaverin formation was clearly reduced by the percentage of 7.7% - 9.3%, 10.4% - 22.9% and 15.8% - 29.8% after addition of clove oil, thyme oil and lemon oil (1%) for 2 days and 4 days (storage time at 4°C).

Table (4) revealed the effect of essential oils (1%) addition on tyramine levels (mg %) in the examined fish samples, in which the mean value of tyramine level in the control group at zero time is 4.38 ± 0.42, while 6.12 ± 0.46 and 7.84 ± 0.61 after 2 days and 4 days (Storage time at 4°C), respectively. Moreover the addition of clove oil (1%) lead to decrease of tyramine levels to the mean value of 5.80 ± 1.50 and 7.01 ± 0.58 after 2 days and 4 days (Storage time at 4°C), respectively, while the addition of thyme oil (1%) decrease the progression of cadaverine levels to the mean value of 5.11 ± 0.45 and 6.56 ± 0.53 after 2 days and 4 days (Storage time at 4°C), respectively. Thus, the addition of lemon oil (1%) gives the highest reduction in cadaverine formation level by the mean value of 4.75 ± 0.41 and 5.87 ± 0.94 after 2 days and 4 days (Storage time at 4°C), respectively. The progression of tyramine formation was clearly reduced by the percentage of 5.2% - 10.6%, 16.5% - 16.3% and 22.4% - 25.1% after addition of clove oil, thyme oil and lemon oil (1%) for 2 days and 4 days (storage time at 4°C).

Traditionally, biogenic amine formation in food has been prevented, primarily by limiting microbial growth through chilling and freezing. However, for many fishing based populations, such measures are not practical. Therefore, secondary control measures to prevent biogenic amine formation in foods or to reduce their levels once formed need to be considered as alternatives. Such approaches to

limit microbial growth may include hydrostatic pressures, irradiation, controlled atmosphere packaging, or the use of food additives(Naila et al. 2010).Essential oils and their components commonly used as flavouring in the food industry also present some antibacterial, antifungal and antioxidant properties (Tajkarimi et al., 2010).

Regarding to histamine, the obtained results are nearly similar to that reported by **[14]** who stated that histamine reduced by addition of thyme oil (1%) from 36.56 ± 1.51 and 52.20 ± 2.79 mg/kg to 32.47 ± 1.51 and41.85 ± 2.04 after 3 days and 7 days (Storage time at 4°C), respectively. Also, similar results were reported by **[15]** who stated that histamine reduced from 21 ± 0.261 mg/kg to 19.08 ± 0.158 mg/kg in luncheon roll meat samples treated by thyme oil extract. Ingestion of food containing small amounts of histamine has little effect on humans, but large amounts histamine can be toxic. The characteristic symptoms of histamine poisoning are rash, urticaria, edema and localized inflammation **[16]**.Such results were higher than that recorded by **[17]** who recorded that The mean values of histamine concentration levels in the control group of chicken meat samples by (mg/kg) in zero time, 3 and 6 days at 4°C were not detected, 4.15 ± 0.025 and 6.54 ± 0.030, respectively, while by addition of Lemon grass oil extract histamine levels were reduced to a mean value of 2.95 ± 0.035, 3.85 ± 0.025 in 3 and 7 days, respectively.

Thyme oil is well known for their antimicrobial activity mainly due to their content of phenolic compounds. The most representative compounds in thyme essential oil was thymol that is structurally having the hydroxyl group at a different location on the phenolic ring The possible mechanism for antimicrobial effect of phenolic compounds include altering microbial cell permeability; interfering with membrane function including electron transport, nutrient uptake, protein and nucleic acid synthesis and enzyme activity; interacting with membrane proteins causing deformation in structure and functionality; and substituting alkyls into phenol nucleus **[18].**

While regarding to putrescine, the present results are lower than that reported by**[14]** who reported that putrescine level in control group at the zero time is 14.70 ± 0.98, and 18.12 ± 1.14 and 24.03 ± 1.95 after 3 days and 7 days (Storage time at 4°C), respectively. Addition of thyme oil (1%) reduced the putrescine level to the mean value of 15.84 ± 1.19 and 22.52 ± 1.77 after 3 days and 4 days (Storage time at 4°C). Also the present results are lower than that reported by **[15]** who reported that the putrescine level decreased from 42.00 ± 0.264 to 33.00 ± 0.173 mg/kg in luncheon roll meat treated with thyme oil extract. Our result are higher than that recorded by **[17]** who recorded that The mean values of putrescine concentration levels in the control group of chicken meat samples by (mg / kg) in zero time, 3 and 6 days at 4°C were not detected, 6.09 ± 0.020 and 8.03 ± 0.025, respectively, while by addition of lemon grass oil extract histamine levels were reduced to a mean value of 3.42 ± 0.025,4.85 ± 0.030 in 3 and 7 days, respectively.

Regarding to cadaverine, the results were higher than that recorded by**[19]** who recorded that cadaverine concentrations (mg/ kg) in control samples of fresh fish fillets of red drum stored for five days at 4°C were 0.90 ± 0.06 and after treatment by clove oil the concentrations were 0.83 ± 0.10.

Concerning to tyramine, the present results are lower than that reported by**[14]** who reported that tyramine was reduced by addition of thyme oil (1%) from 10.78 ± 0.81 and 13.80 ± 1.22 mg/kg to 9.83 ± 0.85 and 11.76 ± 1.07 after 3 days and 7 days (Storage time at 4°C), respectively. Also the present results are lower than that reported by **[17]** who reported that tyramine levels in chicken meat samples were reduced from a mean value of 5.29 ± 0.030 and 7.89 ± 0.010 to 3.85 ± 0.020, 5.94 ± 0.040respectively after addition of lemon grass oil extractfor 3 and 6 days at 4°C.

The antimicrobial activity of the essential oils have been attributed to the presence of some active constituents in the essential oils, mainly the phenolic compounds with a hydroxyl group (-OH). These compounds possess hydrophobic characteristics, which enable them to partition the lipids of bacterial cell membrane and mitochondria and interact with different targets of microbial cell (e.g., cell wall and cytoplasmic membrane), causing loss of cellular constituents, collapse of membrane structure, loss of membrane integrity, dissipation of proton motive force, sequential inhibition of respiration and ion transport processes, impairment of a variety of protective enzymes, involved in the production of energy or synthesis of structural components in microbial cells, possibly through reaction with sulfhydryl compounds or through more non-specific interactions with the protein, alteration in the morphology, structure and function, modification in the transport of nutrients, membrane disruption, extensive leakages from bacterial cells or exit of critical molecules and ions leading to cell death**[20]**.

From the above finding, the essential oil treatment to the fish fillets were effective in retarding fish sensory deterioration, exhibited a positive effect, causing low biogenic amines content. These results indicated that essential oil might be a potential application for extending the shelf life and maintaining the quality of the fish fillets **[21]**.

Table (1) Effect of natural oils (1%) on histamine levels (mg %) in the examined samples of fish fillets (n=5).

Storage time (4°C)	Min	Max	Mean ± S.E*	Progression %	Reduction %
1. Control:					
Zero time	5.3	39.9	26.73 ± 1.16		
2 days	9.6	51.4	32.28 ± 1.40	20.7	
4 days	20.8	68.7	43.91 ± 2.32	64.3	
2. Clove oil (1%):					
Zero time	5.3	39.9	26.73 ± 1.16		
2 days	7.9	47.4	30.52 ± 1.39	14.2	5.5
4 days	15.5	58.2	37.46 ± 1.78	40.1	14.7
3. Thyme oil (1%):					
Zero time	5.3	39.9	26.73 ± 1.16		
2 days	7.4	44.1	29.95 ± 1.31	12.0	7.2
4 days	14.9	51.6	35.12 ± 1.48	31.4	20.0
4. Lemon oil (1%):					
Zero time	5.3	39.9	26.73 ± 1.16		
2 days	6.6	41.4	27.19 ± 1.10	1.7	15.8
4 days	12.4	45.0	31.05 ± 1.22	16.1	29.3

Table (2) Effect of natural oils (1%) on putrescine levels (mg %) in the examined samples of fish fillets (n=5).

Storage time (4°C)	Min	Max	Mean ± S.E*	Progression %	Reduction %
1. Control:					
Zero time	2.0	28.3	17.04 ± 0.82		
2 days	6.7	40.5	22.81 ± 1.39	33.9	
4 days	11.6	57.2	31.97 ± 1.78	87.6	
2. Clove oil (1%):					
Zero time	2.0	28.3	17.04 ± 0.82		
2 days	5.3	35.7	20.63 ± 1.31	21.1	9.6
4 days	7.9	46.1	28.18 ± 1.48	65.2	11.9
3. Thyme oil (1%):					
Zero time	2.0	28.3	17.04 ± 0.82		
2 days	3.7	33.8	19.95 ± 1.17	17.1	12.5
4 days	4.6	40.5	26.72 ± 1.35	56.8	16.4
4. Lemon oil (1%):					
Zero time	2.0	28.3	17.04 ± 0.82		
2 days	2.9	30.1	18.41 ± 1.10	8,0	19.3
4 days	4.2	34.9	21.07 ± 1.24	23.7	34.1

Table (3) Effect of natural oils (1%) on cadaverine levels (mg%) in the examined samples of fish fillets (n=5).

Storage time (4°C)	Min	Max	Mean ± S.E*	Progression %	Reduction %
1. Control:					
Zero time	1.4	16.8	9.57 ± 0.69		
2 days	4.5	23.1	12.03 ± 0.81	25.7	
4 days	6.2	29.3	16.49 ± 1.05	72.3	
2. Clove oil (1%):					
Zero time	1.4	16.8	9.57 ± 0.69		
2 days	4.0	21.2	11.10 ± 0.75	15.3	7.7
4 days	5.6	24.7	14.96 ± 0.98	56.4	9.3
3. Thyme oil (1%):					
Zero time	1.4	16.8	9.57 ± 0.69		
2 days	2.8	19.5	10.78 ± 0.80	12.6	10.4
4 days	3.7	21.9	12.72 ± 1.14	32.9	22.9
4. Lemon oil (1%):					
Zero time	1.4	16.8	9.57 ± 0.69		
2 days	2.5	18.6	10.13 ± 0.72	5.9	15.8
4 days	3.3	20.1	11.58 ± 1.01	21.0	29.8

Table (4) Effect of natural oils (1%) on tyramine levels (mg%) in the examined samples of fish fillets (n=5).

Storage time (4°C)	Min	Max	Mean ± S.E*	Progression %	Reduction %
1. Control:					
Zero time	1.0	9.5	4.38 ± 0.42		
2 days	2.1	12.1	6.12 ± 0.46	39.7	
4 days	2.7	13.5	7.84 ± 0.61	78.9	
2. Clove oil (1%):					
Zero time	1.0	9.5	4.38 ± 0.42		
2 days	1.9	11.0	5.80 ± 0.50	32.4	5.2
4 days	2.4	12.6	7.01 ± 0.58	60.0	10.6
3. Thyme oil (1%):					
Zero time	1.0	9.5	4.38 ± 0.42		
2 days	1.8	10.7	5.11 ± 0.45	16.7	16.5
4 days	2.2	11.5	6.56 ± 0.53	49.8	16.3
4. Lemon oil (1%):					
Zero time	1.0	9.5	4.38 ± 0.42		
2 days	1.5	9.9	4.75 ± 0.41	8.4	22.4
4 days	1.9	10.7	5.87 ± 0.94	34.0	25.1

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