

Reduction of Histamine in Frozen Basa Fish Fillets by Using Essential Oils

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Abstract: A total of 35 samples of frozen fish fillets (*pangasius hypothalamus* “Basa”) was collected from 5 different supermarkets at various localities in Gharbia governorate, Egypt to determine the concentration of histamine level (mg%) before and after treatment by 1% essential oils (thyme, garlic and nigella sativa) using HPLC. The results indicated that the progression of histamine formation was clearly reduced by the percentage of 11.2% and 19.8% with addition of thyme oil(1%) for 3 days and 7 days (Storage time at 4°C), respectively; 13.2% and 23.9% after addition of garlic oil(1%) for 3 days and 7 days (Storage time at 4°C), respectively and the highest reduction level in histamine progression was recorded by the addition of nigella sativa oil (1%) 14.6% and 31.1% for 3 days and 7 days (Storage time at 4°C), respectively. In conclusion, such results can propose that the essential oil treatment to the fish fillets will be helpful in reduction of histamine formation in order to produce fish meat of high quality and safety.

Key words: Histamine • *Pangasius* • Basa • Essential oils • HPLC

INTRODUCTION

Biogenic amines are chemically defined as low molecular weight aliphatic, alicyclic, or heterocyclic organic bases formed from the decarboxylation of amino acids by the metabolic activity of bacteria, plants and animals. Decarboxylation process can proceed through two biochemical pathways: decarboxylation through endogenous (naturally occurring) decarboxylase enzymes or by exogenous decomposition through enzymes released by microflora. The production of amines by the exogenous process is considered more significant [1].

Many biogenic amines have been found in fish, only histamine, cadaverine and putrescine have been found to be significant in fish safety and quality determination. Despite a widely reported association between histamine and scombroid food poisoning, histamine alone appears to be insufficient to cause food toxicity. Putrescine and cadaverine have been suggested to potentiate histamine toxicity. Regardless to spoilage, on the other hand, only cadaverine has been found to be a useful index of the initial stage of fish decomposition [2].

Histidine is converted to histamine by microbial histidine decarboxylase enzyme. Consumption of spoiled fresh, frozen fish and tinned fish products which contain unusually high levels of histamine which results in the outbreaks of histamine fish poisoning is one such food poisoning [3].

The control of biogenic amines formation is mainly focused on the controlling the growth of biogenic amines forming bacteria. Because histamine is heat stable [4]. is not detectable organoleptically by even trained panelists [5]. Once formed, histamine is difficult to destroy by using methods including freezing, cooking, retorting, or smoking [6]. However, there are some methods able to degrade histamine including the gamma irradiation [7] and application of diamine oxidase bacteria to degrade the histamine [8].

This study aimed to investigate the role of certain essential oils (thyme, garlic and nigella sativa oils) as natural bioactive compounds to reduce the concentrations of histamine in frozen Basa fish fillets.

MATERIALS AND METHODS

Within the respect of this study the guidelines adopted by Khalafalla *et al.* [9] were carried out with some modifications. Accordingly, 35 samples of frozen fish fillets (*Pangasius hypophthalmus* "Basa") were bought from 5 different sources (supermarkets and fishmarkets) then classified into 7 groups (5 samples for each group) as follow:

- The 1st group was represented as control group without any additives and stored at 4°C for 7 days.
- The 2nd group was immersed in thyme oil (1%) and stored in refrigerator at 4°C for 3 days.
- The 3rd group was immersed in thyme oil (1%) and stored in refrigerator at 4°C for 7 days.
- The 4th group was immersed in garlic oil (1%) and stored in refrigerator at 4°C for 3 days.
- The 5th group was immersed in garlic oil (1%) and stored in refrigerator at 4°C for 7 days.
- The 6th group was immersed in nigella sativa oil (1%) and stored in refrigerator at 4°C for 3 days.
- The 7th group was immersed in nigella sativa oil (1%) and stored in refrigerator at 4°C for 7 days.

For obtaining essential oils 1%, using tween 80 to dissolve the oil in distilled water according to Hood *et al.* [10].

Actually, 50 grams of each sample were immersed in 100 ml of each essential oil for 15 minutes (El-Captain Company for extracting natural oils, El obour, Cairo, Egypt) under license of Ministry of Health No 33/2006. All controlled and treated samples were analyzed by HPLC for determination of their levels of histamine (mg%) at zero, 3 and 7 days according to the protocol recommended by Krause *et al.* [11] and Pinho *et al.* [12].

RESULTS

The results recorded in Table (1) proclaims the effect of different types of essential oils on histamine levels in the examined basa fish fillet samples, in which the progression% of histamine levels were decreased depending on the type of essential oil and the exposure time.

Results accomplished in Table (2) elaborated the reduction% of histamine levels in the examined basa fish fillet samples among the experimental types of essential oils, in which nigella sativa oil gave the highest percentage in histamine reduction followed by garlic oil then thyme oil.

Table 1: Effect of addition of essential 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%
1. Control:				
Zero time	7.7	56.5	30.68±1.32	
3 days	10.8	64.7	36.56±1.51	19.2
7 days	22.7	92.1	52.20±2.79	70.1
2. Thyme oil (1%):				
Zero time	7.7	56.5	30.68±1.32	
3 days	8.2	61.1	32.47±1.51	5.8
7 days	17.3	70.4	41.85±2.04	36.4
3. Garlic oil (1%):				
Zero time	7.7	56.5	30.68±1.32	
3 days	8.1	58.7	31.72±1.40	3.4
7 days	15.8	68.0	39.73±1.59	29.5
4. Nigella sativa oil:				
Zero time	7.7	56.5	30.68±1.32	
3 days	7.8	58.1	31.22±1.26	1.8
7 days	13.0	62.4	35.96±1.48	17.2

Table 2: Reduction% of histamine levels in the examined samples of fish fillets after application of essential oils (n=5).

Essential oils	Storage time (4°C)	
	3 days	7 days
Thyme oil (1%)	11.2	19.8
Garlic oil (1%)	13.2	23.9
Nigella sativa oil (1%)	14.6	31.1

DISCUSSION

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. Histamine may potentially be degraded by the use of bacterial amine oxidase or amine-negative bacteria. Only some will be cost-effective and practical for use in populations [13].

Essential oils and their components commonly used as flavoring in the food industry also present some antibacterial, antifungal and antioxidant properties [14].

The result achieved in table (1) declared the effect of essential oils (1%) on histamine levels (mg%) in the examined fish fillet samples. In which the mean value of histamine level in the control group at the zero time is 30.68±1.32, while 36.56±1.51 and 52.20±2.79 after 3 days and 7 days (Storage time at 4°C), respectively.

The addition of thyme oil (1%) decreased histamine levels to mean value of 32.47 ± 1.51 and 41.85 ± 2.04 after 3 days and 7 days (Storage time at 4°C), respectively. While by addition of garlic oil (1%) the progression of histamine levels decreased to the mean value of 31.72 ± 1.40 and 39.73 ± 1.59 after 3 days and 7 days (Storage time at 4°C), respectively. Furthermore, the addition of nigella sativa oil (1%) cleared the highest reduction in histamine levels to the mean value of 31.22 ± 1.26 and 35.96 ± 1.48 in 3 days and 7 days (Storage time at 4°C), respectively. Nearly similar results were reported by [Abu-Salem *et al.*, [15] who reported that (histamine was reduced from 21 ± 0.261 mg/kg to 19.08 ± 0.158 mg/kg in luncheon roll meat samples treated by thyme oil extract) and [16] reported that the highest histamine concentration in the control group of fresh fish fillets was 19.41 ± 1.63 mg/kg and the lower level of histamine in essential oils (clove, cumin and spearmint) treated groups were 2.66 ± 0.27 mg/kg, 1.84 ± 0.09 mg/kg and 1.27 ± 0.24 mg/kg, respectively).

Thyme oil is well known for its antimicrobial activity mainly due to their content of phenolic compounds. The most representative compounds in thyme essential oil was thymol, the mode of action of thymol appears to have received the most attention from researchers. Thymol is structurally having the hydroxyl group at a different location on the phenolic ring. The possible mechanism for antimicrobial effect of phenolic compounds includes 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 [17].

Table (2) nominated the reduction% of histamine levels in the examined fish fillet samples, in which the progression of histamine formation was clearly reduced by the percentage of 11.2% and 19.8% with addition of thyme oil (1%) for 3 days and 7 days (Storage time at 4°C), respectively; 13.2% and 23.9% after addition of garlic oil (1%) for 3 days and 7 days (Storage time at 4°C), respectively and the highest reduction level in histamine progression recorded by the addition of nigella sativa oil (1%) was 14.6% and 31.1% for 3 days and 7 days (Storage time at 4°C), respectively.

Garlic was found to be effective in reducing histamine formation and other biogenic amines in fish. However, histamine formation was only reduced by 12% in culture and 9% when garlic was incorporated at 5%. The garlic reduced histamine by inhibiting the growth of histamine-forming bacteria [18].

Antimicrobial activity of garlic could be explained by blocking mechanism by which allicin blocks certain groups of enzymes as cysteine proteinases and alcohol dehydrogenases. These groups of enzymes are found in a wide variety of infectious organisms such as bacteria, fungi and viruses and this provides a scientific basis for broad-spectrum antimicrobial activity of garlic. It is unlikely that bacteria would develop resistance to allicin because this would require modifying several enzymes that make their survival and activity possible [19].

The essential oil of nigella sativa seeds exhibits a broad spectrum of inhibition against many bacterial strains even diluted at 1%. They thus demonstrated that the dithymoquinone present in this oil has a significant antimicrobial activity on bacteria (Gram positive and negative) [20].

The extracts of *N. sativa* showed high inhibitory activity against a range of bacteria resistant to antibiotics. This inhibition is due to the part of the methanolic EO. The methanolic extracts of nigella sativa act by cell wall synthesis inhibition, by inducing changes in membrane structure, by inhibiting bacterial protein synthesis or by binding to ribosomal 50S subunit and interfering with the peptidyl transferase activity [21].

The essential oil treatment to the fish fillets inhibited the microbiological properties throughout the storage periods. All the treatments were effective in retarding fish sensory deterioration, exhibited a positive effect, causing low histamine content, especially histamine, putrescine, cadaverine. These results indicated that essential oil might be a potential application for extending the shelf life and maintaining the quality of the fish fillets [22].

CONCLUSIONS AND RECOMMENDATIONS

The results mentioned above concluded that essential oil treatment of frozen fish fillets is a useful and low cost method to reduce the formation of histamine so that essential oils can be used as natural food preservatives with the freezing method, it will be helpful in reduction of histamine formation in order to produce fish meat of high quality and safety.

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