Current Chemistry Letters 12 (2023) 217–226

Contents lists available atGrowingScience

Current Chemistry Letters

homepage: www.GrowingScience.com

The effect of different treatments on the accumulation of histamine in herring and fesikh

Ibrahim M. Ibrahim[a*], El-Shahat G. El-Dreny[a] and Hind M. Salih[a]

[a]*Special Food and Nutrition Dep., Food Tech. Res. Inst., Agric. Res. Center, Egypt*

C H R O N I C L E

Article history:
Received March 30, 2022
Received in revised form
April 18, 2022
Accepted August 2, 2022
Available online
August 3, 2022

Keywords:
Histamine
Herring
Fesikh
Salted Fish
Toxicity
Mortality
Food Analysis

A B S T R A C T

In Egypt, the request for salt fish products has been increasing during many feasts because it is one of Egypt's favorite foods. It has been reported that in certain seasons, these products cause toxicity and even death. Most fish poisonings around the world are known to be caused by elevated histamine levels. Thus, the study assessed the histamine levels of herring and fesikh samples available on the market and established the safety of these products with recommendations for various treatments that may inhibit the production of histamine in herring and fesikh through bacteria and enzyme production. Before manufacturing herring and fesikh, the fresh fish is soaked for one hour in a modified pH solution of 4 by vinegar with the addition of natural substances individually or in combination (such as garlic, onion, hot pepper, or aloe vera) or some chemicals singly (such as edta, nisin, h2o2, formic acid, and so2). The levels of histamine in herring (111 to 138 mg/kg) and fesikh (214 to 279 mg/kg) were unsafe in marketable samples. The histamine levels of herring and fesikh in proposed treatments were safe since they did not exceed 26 mg/kg after 30 days of cold storage for herring or 45 mg/kg after maturity of fesikh. The proposed treatments enhanced greatly herring and fesikh organoleptic qualities, especially those containing natural ingredients, such as garlic, hot pepper, or their mixture beside onion, which are more accepted treatments.

1. Introduction

Fesikh and herring are conventional Egyptian food connected with particular seasons and celebrations. During the Egyptians' consuming seasons, herring and fesikh have long been a source of numerous poisoning incidents. Symptoms seem withinside the shape of limitation of breath, allergic reaction symptoms, infection, and poisoning that every now and then results in death. Symptoms of histamine poisoning are similar to the ones of an allergic reaction. It is highly suspicious that the primary indicator responsible for these signs is the histamine produced by the bacteria that is found in the fish muscles. Histamine is the primary toxicant in many species of fish. Histamine accumulation causes non-specific gastrointestinal and excess symptoms even after only a few minutes of eating or 24 hr.[1] Consuming it has negative effects on the body, like dilation of peripheral blood vessels, which causes hypotension, headache, and flushing. It is also increased capillary permeability, which causes urticaria, hemoconcentration, edema of the eyelids, smooth muscle contraction (which causes cramps, diarrhea, nausea, and vomiting), rash, face, arms, and neck redness; burning taste; asthma attacks; hives; gastrointestinal symptoms; and swallowing difficulties.[2] Histamine stimulates the heart (raising contractility and causing palpitations and tachycardia), the sensory and motor neurons, causing itching and discomfort. [3] The more vulnerable people whose intolerance or sensitivity to histamine.[4] The different bacterial species can produce histamine by histidine decarboxylase enzyme during handling fish. [5] The activity of this enzyme depends upon pH, temperature, and sodium concentration.[6] Adjusting temperature around melting point inhibited increasing histamine about 50 ppm. [7] As soon as histamine formed, no available treatments for eliminated or destroyed from food.[8] Histamine can form in foods include free histidine or proteins.[9] Both of histamine N-methyltransferase or diamine oxidase enzymes can decomposed histamine. [10] Histamine intolerance induced due a lower activity of diamine oxidase because of the effects of alcohol, drugs,

* Corresponding author.
E-mail address ibrahim256_mah@yahoo.com (I. M. Ibrahim)

doi: 10.5267/j.ccl.2022.8.002

gastrointestinal diseases, or genetic predisposition.[1] Histamine intolerance impacts 1% of people, who are susceptible to this compound.

Histamine hypersensitivity and a range of symptoms can result from diamine oxidase insufficiency.[11] Histamine levels are essential markers of food quality and cleanliness, and a high level is commonly seen as a signal of poor production procedures.[12] Histamine in foods normally are formed as a result of non-controlled microbial action. As a result, numerous governments or organizations have set a regulatory level for histamine in food. Therefore, it was necessary to verify the extent of histamine formation, as well as the concentration formed during the production of herring and fesikh, mainly since they are the most favored foods for Egyptians on many occasions.

Allium plants, such as garlic and onion, are famed as effective in the treatment of infectious diseases, according to.[14] Because of the presence of several hydrophobic antimicrobial components, such as vinyldithiins, allicin, diallyl polysulfides, and ajoenes, garlic has stronger antibacterial activity than other Allium species. The sulfur allicin compound is formed from alliin that raw garlic found has antibacterial properties against both gram positive and negative bacteria. Furthermore, allicin has been demonstrated to inhibit the formation of bacterial biofilms, which is a main reason of microbial resistance to antibiotic treatment.

Onions include quercetin, which has antibacterial capabilities (gram-negative and positive bacteria) and is highly active against the creation of bacterium biofilms.[15]

The medicine and food are used capsaicin extracted from hot peppers for their several actives.[17] Capsaicinoids are widely used in the food and medical industries because they have many useful antioxidant and biological activity,[16] painkiller,[17] anti-flaming,[18] anticarcinoma,[19] and bacteriostatic.[20] Antibacterial action has been demonstrated for volatile pepper.

Aloe vera can use as an anti-bacterial and anti-fungal against diverse strains of pathogenic fungi and bacteria, which may be due to the presence of many substances in the composition such as saponins, enzymes, allucine, aloemodin, aloin, osmanane, amanan, alluride, methyl carmonate, flavonoids, naphtoquinone, sterols, and anthraquinones.[21]

While producing food and medicinal applications, nisin was already being employed as an antibacterial. [22] Nisin's bactericidal effect is caused due to binding with binary lipids at the exterior plasma membrane's surface.[23] It allows holes to make across the cytomembrane, allowing small living material molecules to enter quickly.[24] Nisin has potent antibacterial properties versus gram-positive bacteria but is ineffective against gram-negative bacteria due to not being able to reach the internal membrane.

Because edta acts on the cell surface, it has bacteriostatic properties against both gram-negative and gram-positive bacteria, due to bind to calcium, magnesium, zinc and iron in the outer membrane, leading in external membrane instability and the fast release of approximately half of the lipopolysaccharides and different cell ingrediants.[25]

Sulfur dioxide is among the main important additives used in the world for its antimicrobial and antioxidant properties. A wide range of edible goods may include so_2 as an addition.[26] So_2 is a less costly and more widely used chemical preservative, particularly against lactic and acetic acid bacteria, which produced histamine.[27] Multiple components react with so_2 such as co-factors or enzymes, vitamins, and nucleic acids.[28] The impact of free so_2 on lactic bacteria is more affected by pH.

Furthermore, chemical preservatives such as formic acid and h_2o_2 have been widely utilized to keep fish fresh and reduce the negative consequences of rotting.[29]

Changing the pH to acidic creates unsuitable conditions for many histamine-producing bacteria. It is critical to identify strategies to avoid histamine formation in fish products. As part of the overall improvement in food hygiene during manufacturing and storage, histamine formation must be limited. The primary goal of this study is to determine the safety of herring and fesikh produced in the Egyptian market during seasons and holidays in terms of histamine levels. Additionally, suggest some treatments that may reduce histamine levels in herring and fesikh by incorporating soaking in a pH-modified solution containing natural or chemical additives and reducing water activity by salting process or smoking.

2. Materials and methods

2.1 Material

Fesikh is produced from Egyptian mullet produced at Baltim farms within Kafr El-Sheikh, while Dutch herring is used to make smoked, salted herring. Factories herring samples were brought from Salhia, Faqous (golden lion, M1), Faqous, El-sharkia (tiger, M2), and Gharbia (Anwar, M3). Fesikh samples were brought from the industrial laboratories in Nabarwa, Talkha, Dakahlia [Al Yamani (F1), Kholoud El Nile (F2), and The Pearl (F3)]. All chemicals were of analytical grade and were obtained from Sigma Chem. Co. (St. Louis, MO, USA).

2.2 Methods

2.2.1 Prepare herring and fesikh

Herring was prepared according to the Egyptian standard specifications (288/2016), while fesikh was produced according to the specification (1725/2016), which is technically identical to the international specifications of Codex 244.

2.2.1.1 Herring manufacture

Herring fish are properly washed before being immersed in suitable treatments for even an hour before being allowed to dry for 2 hrs using fans, then the fish were wrapped with coarse salt and placed on a stainless steel mesh for 24 hrs until dry; the excess salt was removed by pieces of clothing. Fish were hanged in the smoking ovens in a vertical position and smoked for 30-40 minutes at temperatures less than 50 °C. Samples were taken for analysis from smoked salted herring after 15, 20, and 30 days of refrigerated storage from the production.

2.2.1.2 Fesikh manufacture

The fish and gills were washed, then immersed for an hr in the recommended treatments before drying for 2-3 hrs under fans. The fish and gills were cleaned, dried beneath fans, and salted (every kilogram of salt has 10 grams of turmeric and 10 grams of dried chili). Every two fish are placed in a stretcher that does not enter the air, and the stretchers are placed in plastic bags with the air removed. Several plastic bags are placed inside a larger bag within a plastic box, and the bags are rotated every day until the fesikh has ripened. The fesikh samples were drawn after 15, 20, and 30 days from ripening and tested.

2.3 Treatments

The different treatments used for herring and fesikh to inhibit bacteria's responsible for the production histamine decarboxylase that removes the carboxyl group of the diamine and the amino acid such as histidine and converts it into histamine, as follow: the fish were soaked 1 hr in diverse treatments.

A: Control treatment washed fish before preparation by water only.

B: Soaking fish for an hr in pH-modified solutions at 4 by vinegar

The treatments are separated into 2 classes:

C: Soaking in a modified pH (4) solution included natural additives

1) 25 g of minced garlic/100 ml (pH_4 minced garlic $_{25\ g/100\ ml}$).
2) 25 g of minced onion/100 ml solution (pH_4 minced onion $_{25\ g/100\ ml}$).
3) 25 g of crushed hot pepper fruits/100 ml solution (pH_4 hot pepper $_{25\ g/100\ ml}$).
4) 10 g of aloe vera/100 ml of solution (pH4 aloe vera $_{10\ g/100\ ml}$).
5) Mixing of 8 g of minced garlic, 8 g of minced onion, 9 g of crushed hot pepper/100 ml solution (pH4 mixed garlic, onion and hot pepper).

D: Soaking in a modified pH (4) solution included chemicals additives

1) 5 ppm edta solution (pH_4 edta $_{5ppm}$).
2) 12 ppm nisin solution (pH_4 nisin $_{12\ ppm}$).
3) 0.6 ml h_2o_2 (30 %) /liter (pH_4 h_2o_2 $_{0.6\ ml/\ liter}$).
4) 100 ppm so_2 solution (pH_4 so_2 $_{100\ ppm}$).
5) 0.3 g formic acid/liter (pH_4 formic $_{0.3\ g\ /\ liter}$)

2.4 Histamine analysis

2.4.1 Standard Preparation

Histamine standard stock solution was prepared at 1 mg/ml methanol and the other concentrations were prepared from it.

2.4.2 Sample Preparation

Five gm of a fish sample (homogenized to fine texture) was placed in flask and completely to 100 ml by methanol 70 %. The solution was treated with ultrasonic radiations for 30 ml in a sonicator. Then, 1 ml of the solution was diluted to 10 ml by methanol and filtered through 0.45 µm filter paper.[30] Histamine is determined in triplicate for each subsample, and the average value of every sample is used.

2.4.3 Equipment

LC-MS/MS containing C-18 column (250 x 4.6 mm) was utilized to assay. The moving phase was comprised of 70% formic acid (0.1): 30% methanol (v/v) with 250 µL flow Rate, and 25µL sample injecting. The whole scan mass range m/z was 50-150 amu, with histamine found at 112 amu. The program Xcalibur was used for the quantitative determination of histamine.

2.5 Sensory analyzes

Ten semi-trained panelists from the members of Food Technology Research Institute, Ismailia, were requested to exhibit their observations from 10 degrees for each criteria of color, odor, taste, appearance, non-black spots, viscera consistency, and texture. Five samples were used in the analysis. The average of the ratings for the test qualities was used to calculate overall acceptability.

2.6 Statistical analysis

Duncan's test, with a significance level of p 0.05, was used to analyze differences between mean values. PASW statistics 18 was used for all statistical procedures (SPSS Inc., USA).

3. Results and Discussion

3.1 Histamine levels in smoked and salted herring after cold storage for15, 20, 30 days

Table 1 discusses the effects of different treatments and storage times at refrigerator temperature on histamine levels in smoked and salted herring. Histamine output in fish may lessen by stopping the action of histamine-generating bacteria or histidine decarboxylase enzymes, which contribute to histamine synthesis.

Table 1. Effect of several treatments on herring histamine levels (mg/kg).

Treatments	After manufacture	15 days	20 days	30 days
Fresh	ND			
Control	ND	ND	15	26
pH_4	ND	ND	ND	ND
M1(golden lion)	35	58	75	125
M2 (Tiger)	42	71	95	111
M3 (Al-Anwar)	46	76	97	138
pH_4 minced garlic $_{25\ g/100\ ml}$	ND	ND	ND	ND
pH_4 minced onion $_{25\ g/100\ ml}$	ND	ND	ND	ND
pH_4 hot pepper $_{25\ g/100\ ml}$	ND	ND	ND	ND
pH_4 aloe vera $_{10\ g/100\ ml}$	ND	ND	ND	ND
pH_4 mixed garlic, onion, and hot pepper	ND	ND	ND	ND
pH_4 edta $_{5\ ppm}$	ND	ND	ND	ND
pH_4 nisin $_{12\ ppm}$	ND	ND	ND	ND
pH_4 h_2o_2 $_{0.6\ ml/\ liter}$	ND	<LOQ	<LOQ	10
pH4 so_2 $_{100\ ppm}$	ND	ND	ND	<LOQ
pH_4 formic $_{0.3\ g\ /\ liter}$	ND	ND	ND	ND

ND (Not detected)
LOQ (Limit of quantitation): 10 ppm

According to the **Table 1**, the fresh fish utilized in the preparation of the treatments in this investigation was histamine-free. The lack of histamine pollution prevents signs of histamine toxicity including rash, redness, vomiting, diarrhea, shortness of breath, tight throat and burning taste. The FDA allows a maximum concentration of 50 ppm.[8] The USFDA considers a level greater than 50 ppm as evidence of decomposition of fish or degraded product. Therefore, the decarboxylase enzymes produced from microorganisms should be avoided during the transportation and storage of raw materials using appropriate and hygienic production conditions (cooling, freezing, salting, etc.). Psychotropic bacteria will grow and liberate histamine in 3 days beneath 10 °C and histamine synthesis takes smaller than 5 days for production by it.[31] It discovered that the best temperature for bacterial decarboxylase synthesis was between 20 and 37 °C.[32] Histamine levels in herring provided by local factories (M1, M2, and M3) immediately after manufacturing were 35, 42, and 45 mg/kg. Histamine levels were not detected in the proposed treatments directly post herring production. The amounts of histamine in the (pH4 h_2o_2 $_{0.6\ ml/liter}$) treatment were less than 10 mg/kg after 15 days of cold storage.

Despite using cold storage conditions for herring samples supplied by local factories, histamine levels in herring after 15 days of processing increased significantly, exceeding 50 mg/kg, which could be due to the use of non-fresh fish, insufficient salting, poor raw material handling and manufacturing procedures. Histamine levels in the suggested treatments remained unchanged after 15 days, and hydrogen peroxide-treated samples were reminded to stay below the 10 mg/kg limit, but the control sample exceeded 15 mg/kg.

When the suggested treatments are compared to those brought from local manufacturers' samples after the 20-day storage period, it is apparent that good manufacturing practice and the freshness of the proposed treatments leads them to have lower concentrations of histamine than the factories samples. It was also clear that the treatments proposed in this research were efficient in lowering histamine levels in herring when compared to control samples; this might be related to garlic, onion, hot pepper, aloe vera, edta, nisin, so_2, and formic acid's capacity to inhibit or destroy the bacteria responsible for histamine production.

It should be noted that the samples provided from the local factories surpassed the acceptable limits after 20 days of storage (50 mg/kg) and reached the not-allowable limits after 30 days (over 100 mg/kg), it could be due to the use of raw materials polluted by histamine. Histamine concentrations surpass the regulation limit of 50 mg/kg, herring is unsuitable for food consumption to deep spoilage;[33] however, it found that histamine concentrations above 50 mg/kg had a normal appearance and odor.[34] The herring storage duration (30 days) relates to the product's circulation in the Egyptian market from production to consumption throughout the seasons of consumption. When the samples brought from the factories were compared to the proposed treatments in this study, whether used for natural or chemical additives, it was discovered that the proportion of histamine in the natural additive treatments did not exceed 10 mg/kg, which is within acceptable limits. This would be due to changed pH by vinegar in all suggested treatments accepting control, which is unsuitable with histidine decarboxylase enzyme activity, as well as the effect of additives utilizing. This helps to explain the low quantity of histamine generated in vinegar-treated herring with a pH of 4. It was observed that the optimal histidine decarboxylase activity by different types of bacteria was between 30 to 40 °C at pH from (6 to 6.5). [35] The reduction in histamine in proposed treatments might be connected to adequate salt, which reduces histamine-creating bacteria, their enzyme production, and activity. [36] Salt reduces food degradation and illness by reducing histamine formation in the product. [37] It discovered that a 5% NaCl concentration lowers histamine production in the culture environment.[38] Excessive NaCl addition, on the other hand, should be avoided,[39] since a limit NaCl consumption (under 5 g/day) is advised to reduce health risks. The cold storage and freezing of fresh fish or herring can reduce histamine production because of a reduction of microbial development and decreased HDC activity,[40] which succeeded in reducing histamine synthesis in some microbes.[41] Furthermore, the data revealed that natural additions are more efficient than chemical additives in avoiding histamine development in herring.

3.2 Sensory evaluation of smoked and salted herring after 30 days of cold storage

The sensory features of preserved smoked herring under cold storage for thirty days and samples brought from different native factories that existed within the same circumstances were displayed in **Table 2**.

Table 2. Effect of various treatments on the sensory characteristics of herring.

Treatments	Color	Odor	Taste	Appearance	NBS	Texture	VC	Overall acceptability
Control	0.6^{e}	8.0^{b}	7.0^{d}	6.0^{d}	0.10^{a}	0.6^{e}	5.0^{e}	6.9i
pH_4	8.0^{c}	10.0^{a}	8.0^{c}	7.0^{c}	10.0a	7.0^{d}	7.0^{c}	8.1^{g}
M1 (Golden lion)	5.1^{g}	7.5^{d}	6.6^{f}	5.7^{f}	9.0^{d}	5.7^{g}	4.7^{h}	6.3^{j}
M2 (Tiger)	4.8^{h}	7.0^{f}	6.0^{g}	5.3^{g}	8.2^{c}	5.2^{h}	4.3^{i}	5.8^{k}
M3 (Al-Anwar)	5.3^{f}	7.8^{c}	6.8^{e}	5.8^{e}	9.5^{b}	5.9^{f}	4.8^{g}	6.6^{l}
pH_4 minced garlic $_{25\ g/100\ ml}$	8.0^{c}	10.0^{a}	10.0^{a}	8.0^{b}	10.0^{a}	9.0^{b}	9.0^{a}	9.1^{b}
pH_4 minced onion $_{25\ g/100\ ml}$	8.0^{c}	10.0^{a}	8.0^{c}	7.0^{c}	10.0^{a}	8.0c	8.0^{b}	8.4^{e}
pH_4 hot pepper $_{25\ g/100\ ml}$	10.0^{a}	10.0^{a}	10.0^{a}	8.0^{b}	10.0^{a}	8.0^{c}	6.0^{d}	8.9^{c}
pH_4 aloe vera $_{10\ g/100\ ml}$	9.0^{b}	10.0^{a}	8.0^{c}	٨,٠^{b}	10.0^{a}	9.0^{b}	6.0^{d}	8.6^{d}
pH_4 mixture	10.0^{a}	10.0^{a}	10.0^{a}	9.0^{a}	10.0^{a}	10.0^{a}	7.0^{c}	9.4^{a}
pH_4 edta $_{5ppm}$	7.0^{d}	10.0^{a}	9.0^{b}	8.0^{b}	10.0^{a}	7.0^{d}	7.0^{c}	8.3^{f}
pH_4 nisin $_{12\ ppm}$	7.0^{d}	10.0^{a}	9.0^{b}	7.0^{c}	10.0^{a}	7.0^{d}	6.0^{d}	8.0^{h}
pH_4 h_2o_2 $_{0.6\ ml/\ liter}$	7.0^{d}	10.0^{a}	10.0^{a}	8.0b	10.0^{a}	9.0^{b}	4.0^{f}	8.3^{f}
pH4 so_2 $_{100\ ppm}$	7.0^{d}	10.0^{a}	9.0^{b}	8.0^{b}	10.0^{a}	9.0^{b}	6.0^{d}	8.4^{e}
pH_4 formic $_{0.3\ g\ /\ liter}$	7.0^{d}	10.0^{a}	8.0^{c}	8.0b	10.0^{a}	7.0^{d}	9.0a	8.4^{e}

pH_4 mixture: solution at pH_4 contain minced garlic, onion, and hot pepper

NBS: Non-black spots, VC: Viscera consistency, M1, M2, and M3: samples brought from different factories.

Concerning color rate, all-natural and chemical treatments outperformed the control sample, which outperformed samples obtained from local manufacturers in terms of improving the color score of herring muscles after cold storage for 30 days. When chemicals and natural additions for herring were evaluated, it was discovered that the latter improved color, especially in the treatments that contained hot pepper alone, garlic, or onion.

Regarding the odor, no differences between the treatments that used natural additives and those that used chemical additives, both of them exceeded the control treatment that exceeded on factory samples.

The use of onions as a natural addition decreases the taste score of herring when compared to the other chemicals and natural additives, but there is no significant difference between it and the control, aloe vera, and formic treatments. Despite the fact that the control treatment excelled in taste at (M1, M2, and M3).

Chemicals and natural additives added in solution soaking improved the look of herring compared to the control sample, with the garlic, onion, and hot pepper mix having the greatest attractiveness. M1, M2, and M3 exhibited lower scores than the control sample.

There were no black spots in any of the suggested treatments evaluated; however, there were several in the factory samples.

When compared to the suggested treatments, the control had the lowest texturing rate. The sample with the greatest rate of texture included a mix of garlic, hot pepper, and onion, whereas the scores for M1, M2, and M3 were quite low.

All recommended treatments enhanced visceral cohesiveness compared to control, with the exception of the h_2o_2 treatment, which was not advised since it caused harm to the herring viscera. Besides, the factory's samples scored worse on viscera consistency than the control, but they exceeded the h_2o_2 treatment.

3.3 Histamine levels in fesikh *after* maturation *for15, 20, 30 days*

Table 3 shows the effect of soaking treatments for one hr in modified pH solutions adjusted at 4 containing natural (minced garlic, minced onion, hot pepper, mixture, and aloe vera) and chemical (edta, nisin, h_2o_2, so_2, and formic acid) additives on the level of histamine in fesikh after maturation for 15, 20, and 30 days at room temperature.

According to **Table 3**, the fresh fish of mullet used in fesikh manufacturing was free of histamine. The raw material's quality and the degree of its low microbial activity, as well as the supply of an environment inappropriate for microbial activity mostly influence the final product's quality and health state.

Solution pH of all recommended treatments to minimize histamine in resulting fesikh was changed to 4 during manufacture, with well salting used during manufacture. A variety of physicochemical parameters, like salt concentration and pH, influence decarboxylase activities, bacterial activity and biogenic amines formation.[43] Salt has regularly added to products to avoid spoiling and food poisoning, which decreases histamine production. When sufficient hygiene conditions, a controlled pH, and a high salt content are satisfied, the formation of biogenic amines is decreased.[42] Furthermore, histamine-producing bacteria can live and develop in low pH environments, and may even release large quantities of histamine in acidic environments.[43]

Table 3. Effect of maturation period and various treatments on fesikh histamine levels (mg/kg).

Treatments	After 15 days	After 20 days	After 30 days
Fresh	ND		
Control	15	25	45
pH_4	ND	ND	<LOQ
F1	139	192	279
F2	117	187	249
F3	٦3	165	214
pH_4 minced garlic $_{25\ g/100\ ml}$	ND	ND	<LOQ
pH_4 minced onion $_{25\ g/100\ ml}$	ND	ND	<LOQ
pH_4 hot pepper $_{25\ g/100\ ml}$	ND	ND	<LOQ
pH_4 aloe vera $_{10\ g/100\ ml}$	ND	ND	12.6
pH_4 mixed garlic, onion, and hot pepper	ND	ND	< LOQ
pH_4 edta $_{5\ ppm}$	ND	< LOQ	10.8
pH_4 nisin $_{12\ ppm}$	ND	ND	<LOQ
pH_4 h_2o_2 $_{0.6\ ml/\ liter}$	<LOQ	<LOQ	10
pH4 so_2 $_{100\ ppm}$	ND	<LOQ	<LOQ
pH_4 formic $_{0.3\ g\ /\ liter}$	ND	ND	<LOQ

ND (Not detected)
LOQ (Limit of quantitation): 10 ppm
F1, F2, and F3: samples brought from different factories.

After 15 days of ripening, the content of histamine in all of the proposed samples, including the control sample, was safe, as the proportion of histamine did not exceed 15 mg/kg. The proportion of histamine in the samples produced by the fesikh manufacturing factories exceeded the permissible levels (139, 117, and 63 mg/kg for samples F1 and F2 and F3), this might be the result of inadequate manufacturing practices. Because excessive histamine levels in most final fish products are mostly caused by microbial contamination, fesikh producers must improve their technology to attain low quantities of critical amines in their products. Mainly since histamine or any biogenic amine, is an indicator of fish deterioration.[44]

All proposed samples (including natural and chemical additives) were collected for histamine determination after 20 and 30 days of maturity. The levels did not surpass the FDA standard (50 mg/kg), although those brought from other companies did. The samples (F1, F2, and F3) exceeded the limits of histamine established (50 mg/kg) by [8] or (100 mg/kg) by [45] or (200 mg/kg) by [46] after 30 days. After maturation for 30 days, F1, F2, and F3 had higher histamine levels of 279, 249, and 214, respectively.

Histamine is considered a vital substance for the body in modest doses since it engages in many physiological processes that the body requires. A high level of histamine in meals, causes a number of problems and harmful repercussions such as foodborne sickness,[47] allergies,[48] multiplication of breast cancer cells,[49] severe pulmonary edema, palpitations, diarrhea, ischemia, headache, myocardial vomiting, respiratory distress, and nausea. As a result, histamine content is regarded as a food quality indicator.

The increase in histamine in the normal control sample (45 mg/kg) after 30 days was caused by inadequate safety against the growth of bacteria that produce the enzyme, which transfers histidine to histamine, or the lack of chemicals or natural preservatives that inhibit the microbes or enzyme's activity.

Histamine levels remained within internationally recommended safe limits by the end of the maturation period in all proposed treatments that used natural (garlic, onions, hot peppers, aloe vera, mixture of garlic, onions, and hot peppers) and chemical (edta, nisin, h_2o_2, so_2, and formic acid) additives in soaking solutions.

3.4 Sensory evaluation of fesikh *after 30 days of maturation*

Table 4 indicates the influence of various soaking treatments containing natural and chemical additives on the sensory qualities of fesikh after a maturing period of 30 days, as well as the sensory qualities of samples presented from other production companies over the same time.

Table 4. Impact of several treatments on fesikh sensory characteristics after 30 days of development

Treatments	Color	Odor	Taste	Texture	VC	Appearance	Overall acceptability
Control	6.0^d	7.0^d	8.0^c	7.0^d	7.0^c	7.0^d	7.0^i
pH_4	7.0^c	7.0^d	8.0^c	8.0^c	7.0^c	7.0^d	7.3^h
F1	5.0^e	6.0^e	5.0^e	6.0^e	7.0^c	6.0^e	5.8^l
F2	6.0^d	6.0^e	7.0^d	6.0^e	7.0^c	5.0^f	6.2^k
F3	7.0^c	6.0^e	7.0^d	6.0^e	8.0b	7.0^d	6.8^j
pH_4 minced garlic $_{25\ g/100\ ml}$	9.0^a	10.0^a	10.0^a	10.0^a	10.0^a	9.0^b	9.7^a
pH_4 minced onion $_{25\ g/100\ ml}$	7.0^c	10.0^a	10.0^a	10.0^a	10.0^a	8.0^c	9.2^b
pH_4 hot pepper $_{25\ g/100\ ml}$	9.0^a	9.0^b	10.0^a	10.0^a	10.0^a	10.0^a	9.7^a
pH_4 aloe vera $_{10\ g/100\ ml}$	7.0^c	7.0^d	10.0^a	9.0^b	8.0b	8.0^c	8.2^f
pH_4 mixture	8.0^b	9.0^b	10.0^a	10.0^a	10.0^a	8.0^c	9.2^b
pH_4 edta $_{5\ ppm}$	9.0^a	7.0^d	10.0^a	8.0^c	10.0^a	8.0^c	8.7^d
pH_4 nisin $_{12\ ppm}$	9.0^a	7.0^d	10.0^a	10.0^a	10.0^a	8.0^c	9.0^c
pH_4 h_2o_2 $_{0.6\ ml/\ liter}$	9.0^a	8.0^c	10.0^a	8.0^c	7.0^c	7.0^d	8.2^f
pH4 so_2 $_{100\ ppm}$	7.0^c	8.0^c	7.0^d	6.0^e	10.0^a	7.0^d	7.5^g
pH_4 formic $_{0.3\ g\ /\ liter}$	5.0e	8.0^c	9.0^b	10.0^a	10.0^a	8.0^c	8.3^e

pH_4 mixture: solution at pH_4 contain minced garlic, onion, and hot pepper
VC: Viscera consistency, M1, M2, and M3: samples brought from different factories.

In terms of color, (it ranges from white - pink - reddish white - light brown to dark brown). Except for the treatment of soaking in a solution containing formic acid (5), which recorded a lower color rate than the control, all natural and chemical treatments used for soaking fish before processing were superior to the control treatment (6) in color, while the treatments brought from factories only F3 (7) significantly improved color corresponded to the control. The color averages in fesikh muscle meat indicate the following colors: average color (5) belonged to dark brown, average color (6) led to light brown, average color (7) varied from pink to reddish-white, average color (8) between pink and white, and average (9) similar to white.

In terms of odor, all of the changed pH treatments that incorporated natural or chemical additives in their solutions gave enhanced odor than the untreated control (7), which displayed no considerable differences, corresponded to the aloe vera or edta treatments. However, the control sample surpassed the factory samples (6).

Almost all treatments using natural ingredients (garlic, onions, hot peppers, or their mix, aloe vera) seemed to have the greatest degree of taste (10), with no significant differences between them and the treatments containing chemical additives such as (edta, h_2o_2 and niacin). The control treatment (8) significantly increased the score of taste compared to F1, F2, and F3, which ranged from (5 to 7).

In terms of texture, it should be emphasized that all of the treatments utilized, whether chemical or natural additions or pH modification, improved the texture of the resulting fesikh compared to untreated (7), which significantly outperformed texture factories grade (6).

In terms of viscera cohesion, there have been no substantial variations between the pH modification alone, h_2o_2, F1 and F2, and control (7), which had the lowest degree of viscera cohesion, however the other treatments notably improved visceral cohesion in contrast to the control.

The appearance of treatments using natural additives demonstrates their superiority, particularly hot pepper, which recorded the greatest appearance, followed by garlic. In general, as compared to the control sample, all recommended treatments improved the appearance, which outperformed on F1 and F2.

In summary, the findings of overall acceptance show that soaking in natural ingredients produces higher quality fesikh than previously soaking in chemical additives. In general, fish soaked in garlic and hot paper solutions produced the best overall acceptability qualities in the resulting fesikh as compared to the control sample, which outperformed the factory's samples.

Fig. 2 summarizes the effect of various treatments on herring and fesikh after 30 days of refrigerated storage of herring or 30 days of ripening for herring. Figures a1 and b1 illustrate that the recommended treatments, either natural or chemical in a pH 4 solution adjusted by vinegar, helped to keep histamine below the unacceptable or hazardous level (50 mg/kg). Figures a2, b2 demonstrate the superiority of the proposed treatments over factory samples in sensory characteristics and acceptance, both herring and fesikh, the three treatments included garlic or hot pepper or the mixing of them with onions were given the highest acceptance.

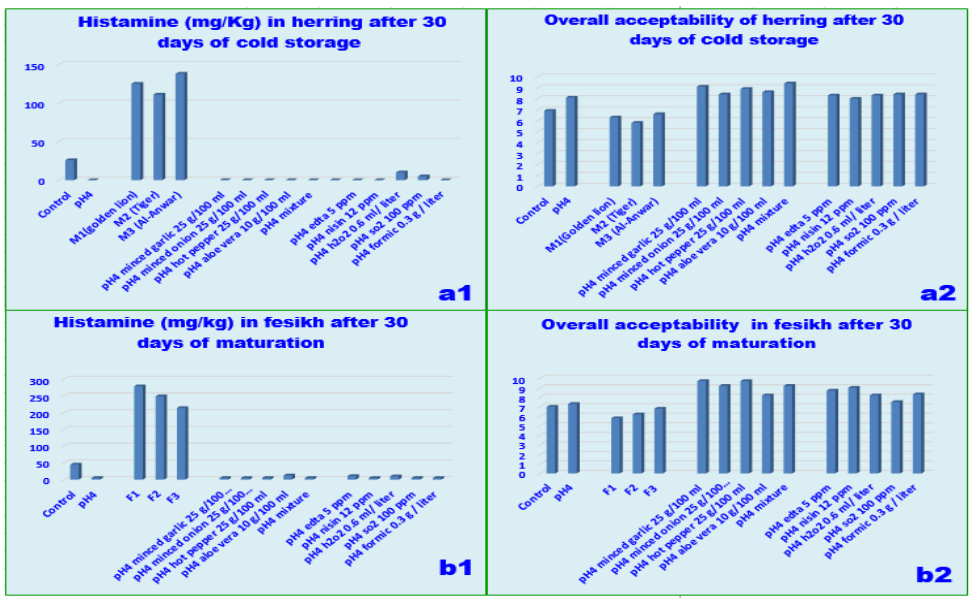

Fig. 2. The impact of several treatments on histamine concentration and acceptance of herring and fesikh after 30 days

4. Conclusion

One of the main concerns in connection with this study is to know the safety of consuming salted fish such as herring and fesikh, which is widely spread on many occasions in Egypt. Moreover, identifying whether histamine is connected to problems of poisoning and death that occur on such occasions is crucial. Most cases of poisoning are treated with gastric lavage, with an analysis of the products that were eaten before the poisoning operations to identify the causes of poisoning, so it was necessary to know the status of some products or brands in the Egyptian market that exist in certain seasons with insufficient control procedures. In addition to searching for some measures and recommendations for producers of such types of fish to prevent the occurrence of poisoning operations or the occurrence of deaths from the consumption of such products. An examination of different salted fish brands available in the Egyptian market showed that herring and fesikh products produced on some occasions are not safe for human consumption. Also, they were proven that the levels of histamine in it exceed the limits of the levels recommended globally, which makes it a strong indication that most cases of poisoning or death resulting from the consumption of these types of fish on such occasions may be due to the high content of histamine. The study proved that soaking fresh fish for 1hr in vinegar solution adjusted at pH 4 with fortified it by natural substances (garlic or onions or hot peppers or aloe vera) or some chemicals compounds before manufacturing herring and fesikh made the levels of histamine in herring and fesikh safe. Also, those treatments, especially those used for natural raw materials (such as garlic, onions, hot peppers, or aloe vera), significantly improved the sensory properties of both herring and fesikh. The sample (pH4 mixed garlic, onion, and hot pepper) was the most acceptable in terms of the results obtained both chemically and sensoryly.

Acknowledgement

The author thanks, all colleagues at the Agricultural Research Center, especially the Food Technology Research Institute.

Formatting of funding sources

The research was conducted at the Agricultural Research Center.

Competing interests

Authors have declared that no competing interests exist.

References

1 Comas-Basté O., Sánchez-Pérez S., Veciana-Nogués M. T., Latorre-Moratalla M. L., and Vidal-Carou M. C. (**2020**) Histamine intolerance: The current state of the art. *Biomolec*., 10 (8) 1181.
2 FAO / WHO (**2012**) joint FAO/WHO expert meeting on the public health risks of histamine and other biogenic amines from fish and fishery products FAO headquarters, Rome, Italy Meeting Report.
3 Knope K. E., Sloan-Gardner T. S., and Stafford R.J. (**2014**) Histamine fish poisoning in Australia, 2001 to 2013 .*Commun. Dis. Intell. Q. Rep*., 38, E285-E293.
4 Benkerroum N. (**2016**) Biogenic amines in dairy products: Origin, incidence, and control means. *Compr. Rev. Food Sci. Food Saf*., 15, 801-826.
5 FAO/WHO. (**2013**) Public health risks of histamine and other biogenic amines from fish and fishery products. Meeting report (9789251078495).
6 Yuan Y., Granger H. J., Zawieja D. C., DeFily D. V., and Chilian W. M. (**1993**) Histamine increases venular permeability via a phospholipase C-NO synthaseguanylate cyclase cascade. *Am. J. Physiol*., 264: H1734-H1739.
7 FDA (**2011**) Fish and fisheries products hazards and controls guide (4^{th} Ed.). Washington, DC: Office of Seafood, Food and Drug Administration, Center for Food Safety and Applied Nutrition, Washington DC.
8 Bremer P. J., Osborne C. M., Kemp R. A., van Veghl P., and Fletcher G. C. (**1998**) The thermal death times of Hafnia alvei in a model suspension and in artificially contaminated hot smoked kahawai (Arripis trutta). *J. Food Prot*., 61, 1047-1051.
9 Tuck C. J., Biesiekierski J. R., Schmid-Grendelmeier P., and Pohl, D. (**2019**) Food intolerances. *Nutrients*, 11 (7).
10 Maintz L., and Novak N. (**2007**) Histamine and histamine intolerance. Amer. *J. Clin. Nutr*, 85 (5) 1185-1196.
11 Ozogul Y., and Ozogul F. (**2020**) Biogenic amines formation, toxicity, regulations in food. In: Saad B., and Tofalo R. (Eds.) Biogenic amines in food: Analysis, occurrence and toxicity. London: Royal Society of Chemistry, 1–17.
12 Galgano F., Favati F., Bonadio M., Lorusso V., and Romano P. (**2009**) *Food Res. Int*., 42:1147.
13 Nakamoto M., Kunimura K., Suzuki J., and Kodera Y. (**2020**) Antimicrobial properties of hydrophobic compounds in garlic: Allicin, vinyldithiin, ajoene and diallyl polysulfides (Review). *Exper. Thera. Medi*., 19, 1550-1553.
14 Sharma K., Mahato N., and Lee Y. R. (**2018**) Systematic study on active compounds as antibacterial and antibiofilm agent in aging onions. *J. food and drug anal*., 26, 518-528.
15 Wang X., Yu L., Li F., Zhang G., and Zhou W. (**2019**) Synthesis of amide derivatives containing capsaicin and their antioxidant and antibacterial activities. *J. Food Bioch*., 43: e13061.
16 Bortolin R. C., Caregnato F. F., Junior A. M., Zanotto-Filho A., Moresco K. S., Rios, A. O., and Moreira J. C. F. (**2016**) Chronic ozone exposure alters the secondary metabolite profile, antioxidant potential, anti-inflammatory property, and quality of red pepper fruit from Capsicum baccatum. *Ecot. Envi. Safe*., 129 (16) 16-24.
17 James K., and Drummond P. D. (**2018**) Rapid induction analgesia for capsaicin-induced pain in university students: A randomized, controlled trial. *Inter. J. Clin. and Expe. Hypn*., 66 (4) 428-450.
18 Zimmer A. R., Leonardi B., Miron D., Schapoval E., Oliveira J. R., and Gosmann G. (**2012**) Antioxidant and anti-inflammatory properties of Capsicum baccatum: From traditional use to scientific approach. *J. of Ethno*., 139 (1) 228-233.
19 Friedman J. R., Nolan N. A., Brown K. C., Miles S. L., Akers A. T., Colclough K. W., and Dasgupta P. (**2018**) Anticancer activity of natural and synthetic capsaicin analogs. *J. Phar. Exper Therap*., 364 (3) 462-473.
20 Bilal M., Rasheed T., Iqbal H. M., Hu H. B., Wang W., and Zhang X. H. (**2017**) Macromolecular agents with antimicrobial potentialities: A drive to combat antimicrobial resistance. *Inter. J. Biolog. Macromolec*., 103, 554-574.
21 Danish P., Ali Q., Haezz M. M., and Malik, A. (**2020**) Antifungal and antibacterial activity of Aloe Vera plant extract. *Biol. Clin. Sci. Res. J*., Volume, 4. https://doi.org/10.54112/bcsrj.v2020i1.4.
22 de Arauz L. J., Jozala A. F., Mazzola P. G., and Penna T. C. (**2009**) Nisin biotechnological production and application: A review. *Tren. Food Sci. Tech*., 20, 146-154.
23 Hasper H. E., Kramer N. E., Smith J. L., Hillman J. D., Zachariah C., and Kuipers O. P. (**2006**) An alternative bactericidal mechanism of action of lantibiotic peptides that target lipid II. *Sci*., 313, 1636-1637.
24 Breukink E., and de Kruijff B. (**2006**) Lipid II as a target for antibiotics. *Natu. Revi. Drug Disc*., 5, 321-323.

25 Umerska A., Strandh M., Cassisa V., Matougui N., Eveillard M., and Saulnier P. (**2018**) Synergistic Effect of Combinations Containing EDTA and the Antimicrobial Peptide AA230, an Arenicin-3 Derivative, on Gram-Negative Bacteria. *Biomole*., 8, 122.

26 Vally H., Misso N., and Madan V. (**2009**) Clinical effects of sulphite additives. *Clin. Exper. Aller*., 39, 1643-1651.

27 Bartowsky E. J. (**2009**) Bacterial spoilage of wine and approaches to minimize it. *Lett. Appl. Microbi*., 48, 149-156.

28 Rose A. H. (**1989**) Transport metabolism of sulfure dioxide in yeasts and filamentous fungi. In: Boddy L., Marchant R., and Read D. J. (Eds.) Nitrogen, phosphorus and sulphur utilization by fungi. Symposium of the British Mycological Society, New York: Cambridge University Press, 59-72.

29 Dembele S., Wang D., Yu L., Sun J., and Dong S. (**2010**) Effects of added crude green tea polyphenol on the lipid oxidation of common carp (Cyprinus carpio L.) and catfish (Clarias gariepinus Burchell) during refrigerated storage. *J. Musc. Foods*, 21, 738-756.

30 AOAC (**2012**) Official method 977.13. Official methods of analysis of AOAC International (19th Ed.). Rockville, MD: AOAC International.

31 Torido Y., Takahashi T., Kuda T., and Kimura B. (**2012**) Analysis of the growth of histamine-producing bacteria and histamine accumulation in fish during storage at low temperatures. *Food Cont*., 26, 174-177.

32 Maijala R. L. (**1993**) Formation of histamine and tyramine by some lactic acid bacteria in MRS broth and modified decarboxylation agar. *Lett. Appl. Micro*., 17, 40-43.

33 Crapo C., and Himelbloom B. (**1999**) Spoilage and histamine in whole Pacific herring (Clupea harengus pallasi) and pink salmon (Oncorhynchus gorbuscha) fillets. *J. Food Safe*., 19, 45-55.

34 Vosikisa V., Papageorgopouloua A., Economoub V., Frillingosc S., and Papadopouloub C. (**2008**) Survey of the histamine content in fish samples randomly selected from the Greek retail market. *Food Addi. Contam*., Part B Vol. 1, 122-129.

35 Kanki M., Yoda T., Tsukamoto T., and Baba E. (**2007**) Histidine decarboxylases and their role in accumulation of histamine in tuna and dried saury. *Appl. Envir. Microb*., 73 (5), 1467-73.

36 Pintado A. I., Pinho O., Ferreira I. M., Pintado M. M., Gomes A. M., and Malcata, F. X. (**2008**) Microbiological, biochemical and biogenic amine profiles of Terrincho cheese manufactured in several dairy farms. *Inter. Dair. J*., 18, 631-640.

37 Linares D. M., Del Río B., Ladero V., Martínez N., Fernández M., Martín M. C., and Álvarez, M. A. (**2012**) Factors influencing biogenic amines accumulation in dairy products. *Fron. Micro*., 3 (180), 1-10.

38 Gardini F., Martuscelli M., Caruso M. C., Galgano F., Crudele M. A., Favati F., and Suzzi, G. (**2001**) Effects of pH, temperature and NaCl concentration on the growth kinetics, proteolytic activity and biogenic amine production of Enterococcus faecalis. *Inter. J. Food Micro*., 64, 105-117.

39 Dotsch-Klerk M., Goossens W. P., Meijer G. W., and Van het Hof K. H. (**2015**) Reducing salt in food; Setting product-specific criteria aiming at a salt intake of 5 g per day. *Euro. J. Clin. Nutr*., 69 (7), 799-804.

40 Santos W. C., Souza M. R., Cerqueira M. M., and Glória, M. B. (**2003**) Bioactive amines formation in milk by Lactococcus in the presence or not of rennet and NaCl at 20 and 32 ◦C. *Food Chem*., 81, 595-606.

41 Díaz M., del Río B., Redruello B., Sánchez-Llana E., Martin M. C., Fernández M., and Ladero V. (**2018**) Lactobacillus parabuchneri produces histamine in refrigerated cheese at a temperature dependent rate. *Inter. J. Food Sci. Tech*., 53(10), 2342-2348.

42 Valsamaki K., Michaelidou A., and Polychroniadou A. (**2000**) Biogenic amine production in Feta cheese. *Food Chem*., 71, 259-266.

43 Barbieri F., Montanari C., Gardini F., and Tabanelli, G. (**2019**) Biogenic amine production by Lactic Acid Bacteria: A Review. *Foods*, 8 (1) 1-37.

44 Biji K. B., Ravishankar C. N., Venkateswarlu R., Mohan C. O., and Srinivasa Gopal T. K. (**2016**) Biogenic amines in seafood: a review. *J. Food Sci. Tech*., 53(5), 2210-2218.

45 EU Directive, Regulation (EC) no 1441/2007 of 5 December 2007, Off. J. Eur. Union (**2007**) L 322/12-29.

46 Regulation (EU) 2015/2283 of the European Parliament and of the Council of 25 November (**2015**) on novel foods, amending Regulation (EU) No 1169/2011 of the European Parliament and of the Council and repealing Regulation (EC) No 258/97 of the European Parliament and of the Council and Commission Regulation (EC) No 1852/2001

47 Ismail A. B., Susan P., Hilton C. D., and Gary A. D. (**2009**) Biogenic amines in fish: roles in intoxication, spoilage, and nitrosamine formation–a review. *Crit. Rev. Food Sci. Nutr*., 49, 369-377.

48 Chen H. C., Hwang D. F., Chiou T. K., and Tsai Y. H. (**2011**) Determination of histamine in mahi-mahi fillets (Coryphaena hippurus) implicated in a foodborne poisoning. *J. Food Saf*., 31, 320-325.

49 Satoshi T., Minoru S., Hiroki Y., Yoshihide U., and Shinya H. (**2016**) Histamine H3 receptor antagonist OUP-186 attenuates the proliferation of cultured human breast cancer cell lines. *Biochem. Biophys. Res. Commun*., 480, 479-485.