Investigating histamine levels, microbial and chemical properties in industrial and traditional drying methods of anchovy fish in Qeshm Island

Abstract

Histamine or scombroid food poisoning can occur due to the consumption of high levels of histamine in fish and cause physiological disorders in humans. Since the Persian Gulf is the main food source of anchovy fish, it may be contaminated with high histamine levels. Therefore, this study was conducted to investigate changes in histamine levels, microbial and chemical properties in Persian Gulf anchovies using two industrial and traditional drying methods and comparing the differences between these methods. After sampling and preparation, peroxide value (PV), total volatile basic nitrogen (TVB-N), microbial tests, sensory evaluation and measuring the amount of histamine were performed using high-performance liquid chromatography (HPLC). The results of the present study showed that histamine levels increase during the drying process. Traditional method results no higher histamine levels than the industrial method, So that the amount of histamine was reported in fresh fish 3215 mg/kg, traditional dried samples 766 mg/kg and in industrial dried samples 764 mg/kg respectively, and no significant difference was observed in the measured histamine levels between the two drying methods ($P > 0.05$). Still, the amount of histamine in both methods was significantly lower with fresh fish ($P < 0.05$). On the other hand, there was a significant difference in the amount of TVB-N of the samples ($P<0.05$), and the highest amount was related to the samples dried by the traditional method and the lowest amount was reported in the fresh fish samples. Also, there was a significant difference in the amount of PV of the samples ($P<0.05$)**.** Findings suggest that the measurable concentration of histamine in fish products may vary depending on the; fishing methods, fishing season, fish size, temperature and type of drying process, rate of histamine production and the decomposition rate during preparation and drying.

Keywords: Food poisoning, histamine, fish, drying

1. Introduction

The coastal waters of Hormozgan province are home to a population of small surface fish in the Persian Gulf, with anchovy fish being particularly important. Anchovies belong to the "Engraulidea" family and are distributed in the order "Clupeiformes". They are a major component of the small-scale fish catch in the Persian Gulf and Oman Sea each year. Additionally, anchovy fish play a crucial role in the food cycle of marine ecosystems, serving as a food source for larger fish and occupying an important ecological niche in the seafood cycle (1).

Histamine is a significant biogenic amine found in fish tissue that has gained attention due to the symptoms of poisoning that can occur after consuming relatively large amounts of this substance (2). Other biogenic amines, such as cadaverine and putrescine, which are present in decaying aquatic animals, may also contribute to the effects of histamine. Histamines and other biogenic amines, such as cadaverine and agmatine, can affect the quality of tuna stored under ice (2). Histidine is a non-protein nitrogen derivative found in fish and other seafood, with higher levels in various fish species, including anchovies, than in other food products. Some bacteria, which are the natural flora of these products, convert histidine to histamine. Histamine-producing bacteria (HPB) such as *Photobacterium phosphoreum* and *Raoultella planticola* possess histidine decarboxylase (HDC), which converts histidine into histamine (3, 4).

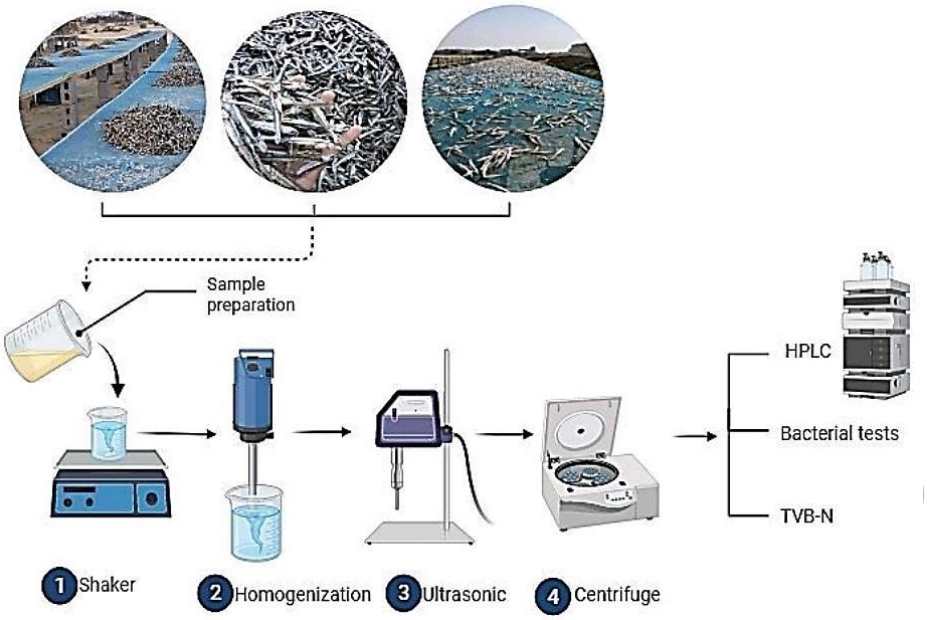
Most histamine-producing bacteria are not naturally present in the skin, bronchi, and viscera of fish, but are transmitted through secondary contamination after capture, handling, processing, and sale (3). In fish, histamine levels increase as the fish begins to break down. Unfortunately, there is limited information about the conditions that cause these substances to accumulate in fish and be transmitted to humans. The type and number of substances that exacerbate histamine poisoning depend on various factors, such as the fish species, storage time and temperature, type of fish microbial flora, and their metabolic abilities (5, 6).

Exposure of fresh fish to high temperatures can lead to an increase in the growth of histidine decarboxylase-producing bacteria, resulting in elevated histamine levels (7, 8). Additionally, the literature has reported that the purity of the ice used to store fish in the refrigerator is also crucial, as it can be a source of histamine-producing bacteria (7, 8). Although various factors affecting histamine levels in fish have been studied, the impact of drying a popular method for fish storage, on histamine levels has not been thoroughly investigated. Therefore, we conducted a study to monitor changes in histamine levels during the drying process under different conditions.

2. Materials and methods

2.1. Sample collection and preparation

First, 100 samples of each group (fresh, industrial, and traditional dried fishes) entered the laboratory and was ground and homogenized (Picture 1). Then 10 mL of perchloric acid solution was added to it. The sample mixture was stirred with a shaker (10 minutes), ultrasonic (10 minutes) then centrifuged (12 minutes) at 4°C/12000 rpm. The supernatant was passed through a needle filter, and after the injection of working standards and QC sample, 20 µl was injected into the HPLC (7, 8).



Picture 1. Sample preparation and methods

2.2. Histamine measurement by HPLC

For this purpose, 20 microliters of extract were injected into an 8C reverse phase column with a length of 250 mm, an internal diameter of 6.4 mm, and a particle diameter of 5 micrometers. The injection conditions were set isocratically. The detector of the HPLC was chosen as fluorescence and this detector was set at the excitation wavelength of 343 nm and the emission wavelength of 445 nm. The temperature of the column was set at 40°C. Mobile phase with a 0.4 ml/min speed by a pump connected to the HPLC device passed through the column. The mobile phase consists of solutions A and B, respectively. Solution A contains 4.5 buffer containing 0.01 M phosphate and sodium 1-decane sulfonate 0.002 M and solution B contains ultrapure acetonitrile. The mobile phase was prepared by combining 80% of solution A and 20% of solution B. The volume of each injection into the device was 2 microliters (7, 8).

2.3. Microbial properties

Fishes were processed for 60 seconds in a Blender. A mixture of 25 gr of ground sample and 225 mL of 0.1% peptone (Merck, Germany) in 0.85% bacteriological sodium chloride (Merck, Germany) was mixed. 0.1 mL from each series of dilutions of anchovy suspension was cultured on tryptone soya agar (TSA).

2.3.1. Coliforms count

Violet-Red Bile Agar (Ibresco, Iran) was utilized to enumerate coliform bacteria. The plates were aerobically incubated at 37°C for two days, and circular, purplish-pink colonies with a diameter of 1 to 2 mm and encircled by purple halos were identified as coliforms (9-11).

2.4. Total volatile basic nitrogen (TVB-N)

The Total volatile basic nitrogen content of the samples was measured by the macrocaldal method and the amount of volatile bases was calculated in terms of mg percentage from the following equation (12-14).

$$\text{Total volatile basic nitrogen (\%)} = 100 \times 14 \times 0.1 \text{ acid}$$

2.5. Peroxide value (PV)

To measure the peroxide value, the fat of the samples was separated and then the amount of iodine released by titration and the amount of peroxide was calculated according to AOCS (1990) (15-17).

2.6. Sensory evaluation

In order to check the sensory and organoleptic characteristics of the sample, a panel of 5 people, whose members were educated people present in the laboratory, was used, and for evaluation, a three-point hedonic scoring system was performed (7, 8).

2.7. Data analysis and statistics

We used SPSS version 18 statistical software was used to analyze the data. T-test, ANOVA, and other tests were utilized as appropriate (18, 19).

3. Results

3.1. Histamine changes in fish

Both industrial and traditional methods changed the amount of histamine measured in fish expected in comparison with fresh fish. The results showed that the amount of histamine measured in fish samples dried traditionally was close to the industrial method (Fig 1). So that the amount of histamine was reported in fresh fish 3215 mg/kg, traditional dried samples 766 mg/kg and in industrial dried samples 764 mg/kg respectively, and no significant difference was observed between traditional and industrial methods ($P > 0.05$), but the amount of histamine in both methods was significantly lower with fresh fish ($P < 0.05$).

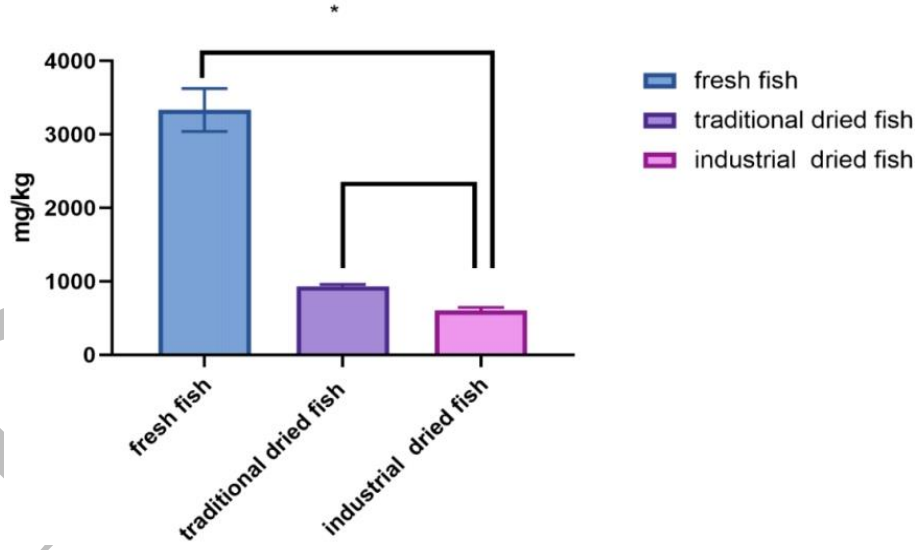


Figure 1. Changes in measured histamine (mg/kg) among fresh, traditional, and industrial dried fish (*$P < 0.05$)

3.2. Bacterial count

As histamine is a product of bacteria activity we count the number of bacteria in fresh, traditional, and industrial dried fishes. Results showed that the bacteria number in fresh samples is higher than in dried fish regardless of their preparation method (Fig 2). In addition, we compared the number of bacteria in traditional and industrial dried samples which show a low number of bacteria in industrial. The coliform bacteria have not been detected in any of the samples.

The correlation of bacteria count versus histamine level was observed, confirming the link of bacteria production of histamine in samples (Fig 3).

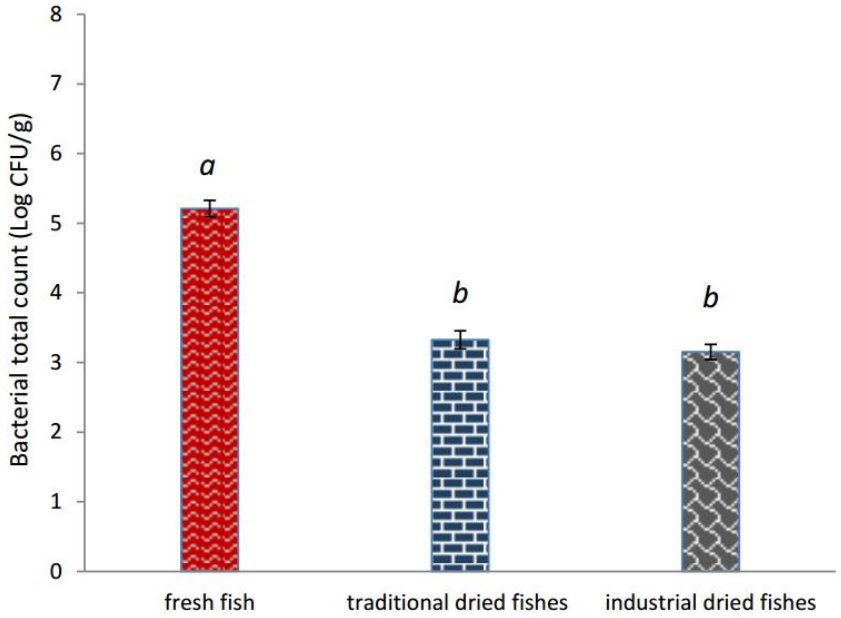


Figure 2. changes in bacterial total count (Log CFU/g) among fresh, traditional, and industrial dried fish. Non-similar lower case letters indicate significant differences between groups (P<0.05).

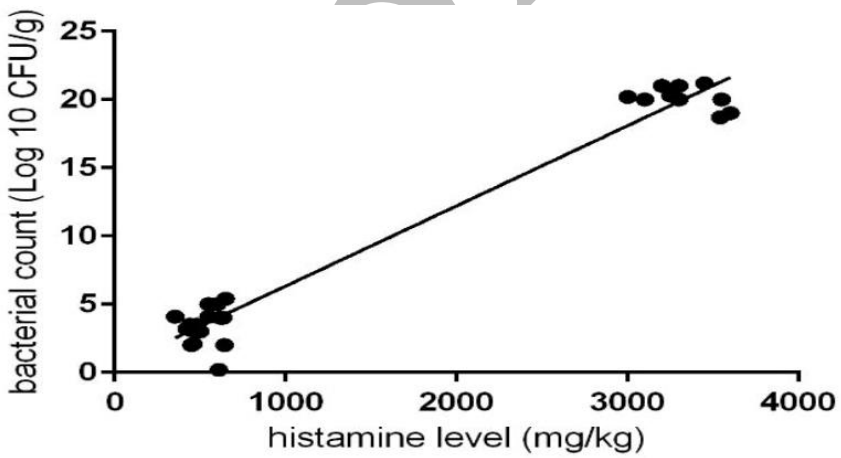


Figure 3. Correlation of bacterial total count and histamine among fresh, traditional, and industrial dried fish (*P> 0.05)

3.3. Peroxide value (PV) and Total volatile basic nitrogen (TVB-N)

Changes in Peroxide value and volatile basic nitrogen content in samples are shown in Fig 4. The amount of TVB-N in fresh, traditional, and industrial dried fish were 64.92, 89.04 and 77.49 mg/100 g respectively. According to the results, there was a significant difference in the amount of TVB-N of the samples (P<0.05), and the highest amount was related to the samples dried by the traditional method and the lowest amount was reported in the fresh fish samples. Also, there was a significant difference in the amount of PV of the samples (P<0.05). So in fresh, traditional, and industrial dried fish were 0.92, 1.69 and 1.41 meq/kg respectively.

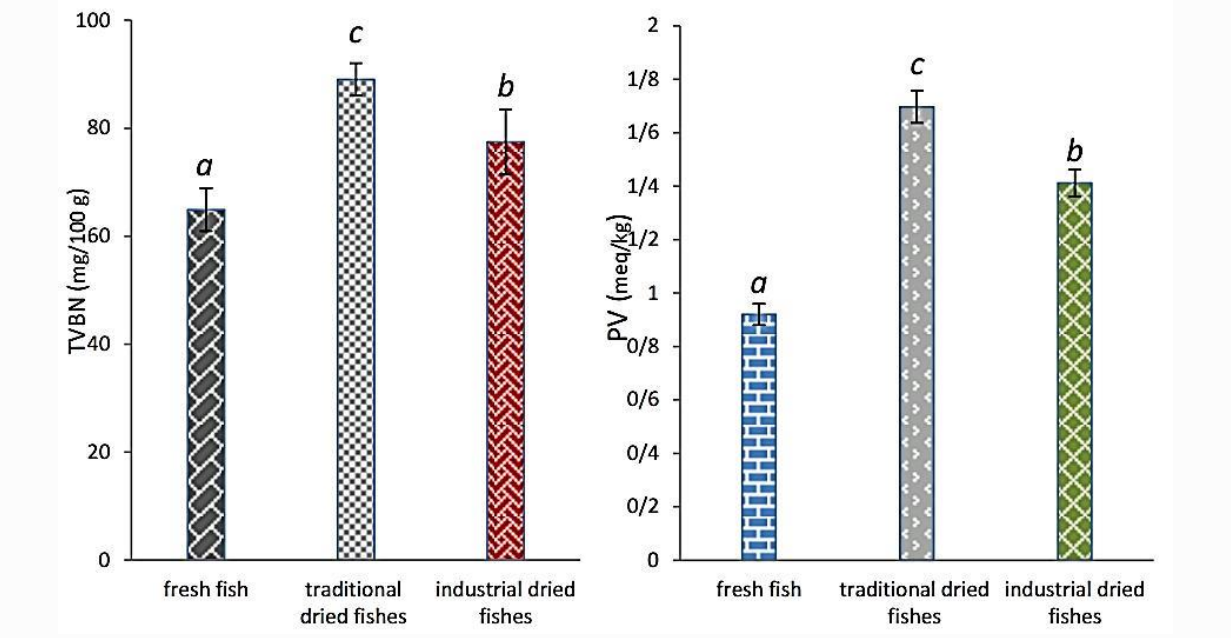


Figure 4. Changes in TVB-N and PV in different groups. Non-similar lower case letters indicate significant differences between groups (P<0.05).

3.4. Sensory evaluation

Changes in sensory characteristics (taste, aroma, color, texture and overall acceptance) of samples are reported in Fig 5. According to the results shown, industrial dried fish samples had more aroma, texture, and overall acceptance scores compared to other groups (P<0.05), while in the evaluation of taste and color, the highest score was related to the fresh fish samples (P<0.05).

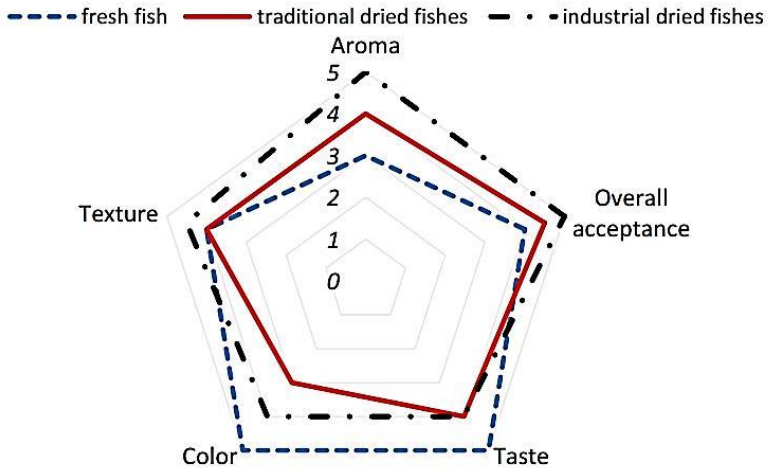


Figure 5. Sensory evaluation results of different groups

4. Discussion

The primary objective of this study was to investigate the changes in histamine levels in Anchovy fish on Qeshm Island using both industrial and traditional drying methods, as well as the effect of different methods on bacterial flora. Findings revealed that although the range of changes in histamine levels measured in both industrial and traditional methods was low, the trend of changes related to the average showed that the histamine levels measured in traditional and industrial methods were

significantly higher than fresh fish. This indicates that the traditional drying method can also be effective in reducing fish histamine levels.

According to the approval of the United States Food and Drug Administration (FDA), aquatic products containing more than 9 mg/100 g of histamine are unsuitable for human consumption, and regulatory bodies are required to prevent their sale (20). Marques *et al.,* (2019) have reported that the amount of histamine accumulation in different aquatic species is different There is a serious risk in similar conditions in terms of histamine production and the occurrence of poisoning with it in some species, while this may not be the case in some other species and the amount of histamine is at an acceptable level (21).

Yesudhason *et al.,* (2013), in a study, the amount of histamine in fresh, frozen, canned and dried fish samples of scombroid and non-scombroid species of Oman using a high-performance liquid chromatography with a fluorescence detector. According to the results, histamine was detected at a rate of about 41.8% among fresh fish, 61.0% of frozen fish, 78.9% of canned fish and 91.6% of dried fish samples. Which is different from the results of the present study, this could be because the amount of histamine in dried fish depends on factors such as; fishing methods, fishing season, fish size, temperature and type of drying process (22). They also stated that imported dried anchovies contained high histamine levels. The study confirms that post-catching and commercialisation practices of seafood are adequate, warranting good quality fish and may not cause histamine risk to consumers in terms of human diet, while necessary monitoring may be done for imported dried fish products (22).

Furthermore, the results of bacterial counts were higher in fresh fish and lower in dried fish, with the lowest growth observed in dried fish processed using the industrial method. This suggests that the industrial drying method may be more effective in reducing bacterial growth in fish compared to the traditional method. As expected, bacterial count and histamine level were related which shows bacterial count number in histamine level.

Rodtonga *et al.,* (2005) while investigating the amount of psychrophilic bacteria in their effect on increasing the amount of histamine in Indian moto fishes, pointed out that the highest amount of histamine of 13.3 mg/200 grams was obtained within 40 h after storage, and it greatly increased with the increase in temperature 35°C increased, which shows the effect of temperature on the increase of histamine (23).

Islam *et al.,* (2012) In a study, investigated the nutritive and food qualities of traditional, rotary and solar tunnel dried mola (Amblypharyngodon mola), They announced that the values of total volatile basic nitrogen (TVB-N) from 10.64 to 20.36 mg/100 g were found to differ among the groups and the highest amount was related to samples dried by the traditional method which is consistent with the results of the present study (24).

Tenyang *et al.,* (2020) investigated the effects of traditional and industrial drying methods on lipid oxidation and fatty acid composition of two species of freshwater fish. Their results showed that these two methods significantly accelerate lipid oxidation by increasing the amount of peroxide and the amount of total oxidation. These results are consistent with the findings of the present study (25).

In a study, Sensory characteristics, nutritive and food qualities of traditional, rotary and solar tunnel dried mola (Amblypharyngodon mola) products were investigated (24). Organoleptically, the color of traditional dried mola products was slightly brownish with the emission of a faint off aroma. On the other hand, the color of dried mola fish produced in rotary and solar tunnel dryers showed a characteristic shiny color and whitish to slight brownish respectively, but it received fewer points from fresh samples. For traditional dried mola products texture was somewhat soft, whereas it was firm and flexible with characteristic fresh fishlike aroma for rotary dryers and solar tunnel dryers. Traditionally dried products were not satisfactory which is consistent with the results of the present study.

Given the importance of histamine levels in edible fish and the increasing demand for fish as a healthy and common food source, monitoring histamine levels in fish is crucial. This study highlights the need for further research to investigate the most effective methods for reducing histamine levels in fish and to ensure the safety and quality of fish products for human consumption.

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Authors' Contribution

Amin Dara collected the samples and conducted the experimental work, Afshin Akhondzadeh Basti and Asghar Azizian Analysis and interpretation of data, Peyman Mahasti shotorbani, Saeid Tamadoni Jahromi and Mehdi Jabar Zadeh shiadeh writing and revising the manuscript.

Ethics

The authors of this study declare that all steps in this study, carried out in accordance with the principles of the ethics committee of the Iranian Veterinary Organization.

Conflicts of Interest

The authors declare that they have no conflicts of interest

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

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