Original Article

Investigating Histamine Levels, Microbial and Chemical Properties in Industrial and Traditional Drying Methods of Anchovy Fish in Qeshm Island

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ABSTRACT

The occurrence of histamine, or scombroid food poisoning, can be attributed to the ingestion of elevated levels of histamine in fish, resulting in physiological disturbances in humans.Given that the Persian Gulf is a primary anchovy fish food source, it is plausible that the fish may be contaminated with high histamine levels.Consequently, this study was undertaken to examine alterations in histamine levels, microbial and chemical characteristics in Persian Gulf anchovies subjected to two industrial and traditional drying methods, and to contrast the disparities between these methods. The experimental design involved the collection and preparation of samples, followed by the measurement of peroxide value (PV), total volatile basic nitrogen (TVB-N), microbial tests, sensory evaluation, and the quantification of histamine using high-performance liquid chromatography (HPLC).The findings of the present study demonstrated that the levels of histamine increased during the drying process. The findings revealed that the traditional method did not yield higher histamine levels than the industrial method, with the amount of histamine reported as 3215 mg/kg in fresh fish, 766 mg/kg in traditional dried samples, and 764 mg/kg in industrial dried samples, respectively. Moreover, no significant difference was observed in the measured histamine levels between the two drying methods ($P>0.05$). However, a significant decrease in histamine levels was observed in fresh fish samples in comparison to both drying methods ($P<0.05$).Conversely, a significant variation in the levels of TVB-N was detected among the samples ($P<0.05$), with the highest levels observed in samples subjected to the traditional method and the lowest levels detected in fresh fish samples. Furthermore, a significant difference was observed in the amount of PV of the samples ($P<0.05$).The findings of this study suggest that the measurable concentration of histamine in fish products may vary depending on several factors, including 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 (Engraulis encrasicolus) being of particular importance. 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. 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, a significant biogenic amine found in fish tissue, 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, a non-protein nitrogen derivative found in fish and other seafood, has been found to be present in higher concentrations in various fish species, including anchovies, than in other food products (5). Some bacteria, which are the natural flora of these products, have been shown to convert histidine to histamine. Histamine-producing bacteria (HPB), such as Photobacterium phosphoreum and Raoultella planticola, possess histidine decarboxylase (HDC), an enzyme that catalyses the decarboxylation of histidine to histamine (3, 4). The majority of histamine-producing bacteria are not naturally present in the skin, bronchi, and viscera of fish; rather, they 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 exacerbating factors for such poisoning are multifaceted, including the fish species, duration and temperature of storage, the microbial flora of the fish, and their metabolic abilities (5, 6). Exposure of fresh fish to elevated temperatures has been shown to increase the growth of histidine decarboxylase-producing bacteria, resulting in elevated histamine levels (7, 8). The literature has reported that the purity of the ice used to store fish in refrigerators 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

Firstly, 100 samples of each group (fresh, industrial, and traditional dried fish) were entered into the laboratory and ground and homogenized (Figure 1). Then, 10 mL of perchloric acid solution was added to the samples, which were stirred with a shaker (10 minutes), ultrasonic (10 minutes), then subjected to centrifugation (12 minutes) at 4°C/12,000 rpm. The resultant clear upper layer was then passed through a needle filter. Following the injection of working standards and QC sample, 20 µl of the sample was injected into the HPLC (7, 8).

2.2. Histamine Measurement by HPLC

For the present study, 20 microliters of extract were injected into an 8C reverse phase column (250 mm in length, with an internal diameter of 6.4 mm and a particle diameter of 5 micrometers). The injection conditions were set isocratically, and the detector of the HPLC was chosen as fluorescence. 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, and the mobile phase, consisting of solutions A and B, was passed through the column at a speed of 0.4 ml/min by a pump connected to the HPLC device. 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

The fish samples were subjected to a 60-second processing cycle in a blender. A mixture of 25 g of ground sample and 225 mL of 0.1% peptone (Merck, Germany) in 0.85% bacteriological sodium chloride (Merck, Germany) was prepared. 0.1 mL from each series of dilutions of anchovy suspension was cultured on tryptone soya agar (TSA).

2.3.1. Coliforms Count

Coliform bacteria were enumerated using Violet-Red Bile Agar (Ibresco, Iran). The plates were subjected to aerobic incubation at 37°C for a period of two days, after which 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 milligrams percentage from the following equation (12-14).

Total volatile basic nitrogen (%) = 100 × 14× 0.1 acid

2.5. Peroxide Value (PV)

The peroxide value was measured by subjecting the fat samples to a separation process, followed by the calculation of the amount of iodine released during the titration and the subsequent determination of the peroxide content, as outlined in AOCS (1990) (15-17).

2.6. Sensory Evaluation

In order to verify the sensory and organoleptic characteristics of the sample, a panel of five individuals was employed. The members of the panel were educated and

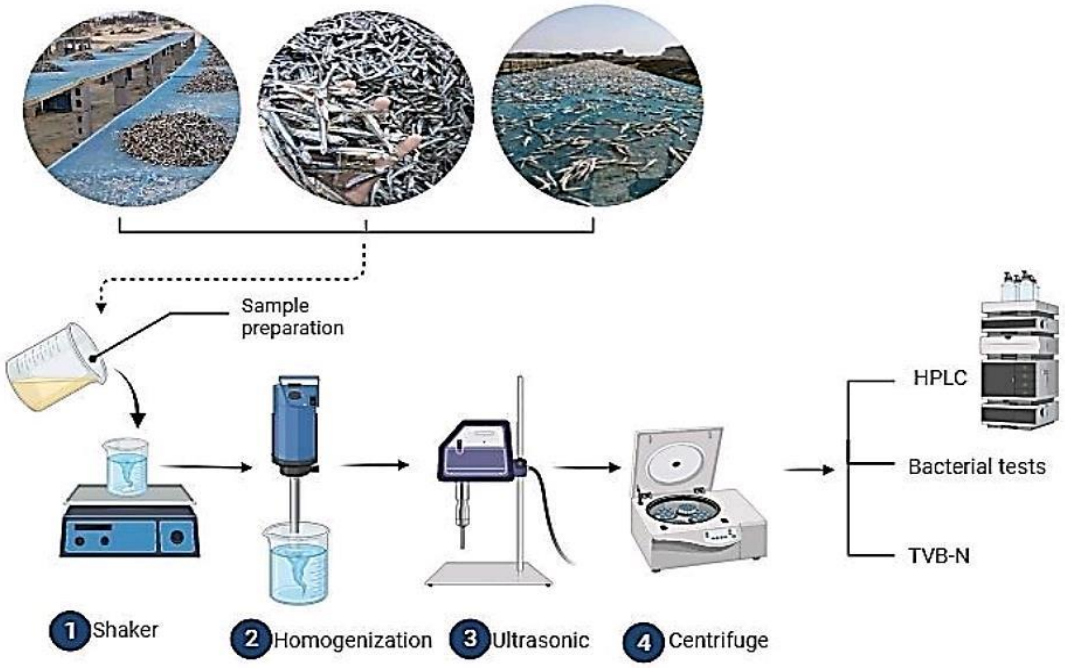

Figure 1. Sample preparation and methods.

present in the laboratory. A three-point hedonic scoring system was utilized for the evaluation process (7, 8).

2.7. Data analysis and statistics

The analysis of the data was conducted utilising SPSS version 18 statistical software. The T-test, ANOVA, and other relevant tests were employed as deemed necessary (18, 19).

3. Results

3.1. Histamine Changes in Fish

The study revealed that both industrial and traditional methods resulted in alterations to the measured levels of histamine in fish samples when compared with fresh fish. The findings demonstrated that the amount of histamine measured in fish samples dried using traditional methods closely approximated the levels observed in industrial samples (Figure 2). The histamine content was reported as follows: 3215 mg/kg in fresh fish, 766 mg/kg in traditional dried samples, and 764 mg/kg in industrial dried samples. No significant difference was observed between the traditional and industrial methods ($P>0.05$), but the amount of histamine in both methods was significantly lower in fresh fish ($P<0.05$).

3.2. Bacterial Count

As histamine is a product of bacterial activity, the number of bacteria in fresh, traditional, and industrial dried fish samples was enumerated. The results demonstrated that the bacteria count in fresh samples was higher than in dried fish, irrespective of their preparation method (Figure 3). Furthermore, a comparison was made of the number of bacteria in traditional and industrial dried samples, which exhibited a low number of bacteria in the latter. Coliform bacteria were not detected in any of the samples. The correlation between bacteria count and histamine level was observed, confirming the association between bacterial production of histamine in samples (Figure 4).

3.3. Peroxide Value (PV) and Total Volatile Basic Nitrogen (TVB-N)

As demonstrated in Figure 5, a clear distinction is evident in the alterations of peroxide value and volatile basic nitrogen content among the various samples. The levels of TVB-N in fresh, traditional, and industrial dried fish were recorded as 64.92, 89.04, and 77.49 mg/100 g, respectively. A statistically significant difference ($P<0.05$) in the amount of TVB-N was observed among the samples, with the highest and lowest amounts recorded in the samples dried by the traditional method and the fresh fish samples, respectively.Additionally, a statistically significant difference ($P<0.05$) in the amount of PV was observed among the samples. The levels of PV were found to be 0.92 meq/kg in fresh fish, 1.69 meq/kg in traditional dried fish, and 1.41 meq/kg in industrial dried fish.

3.4. Sensory Evaluation

As demonstrated in Figure 6, a series of alterations in sensory characteristics (including taste, aroma, colour, texture and overall acceptance) were observed among the samples. The findings indicate that industrial dried fish samples exhibited higher levels of aroma, texture and overall acceptance in comparison to the other groups ($P<0.05$). Conversely, in the assessment of taste and colour, the highest scores were attributed to the fresh fish samples ($P<0.05$).

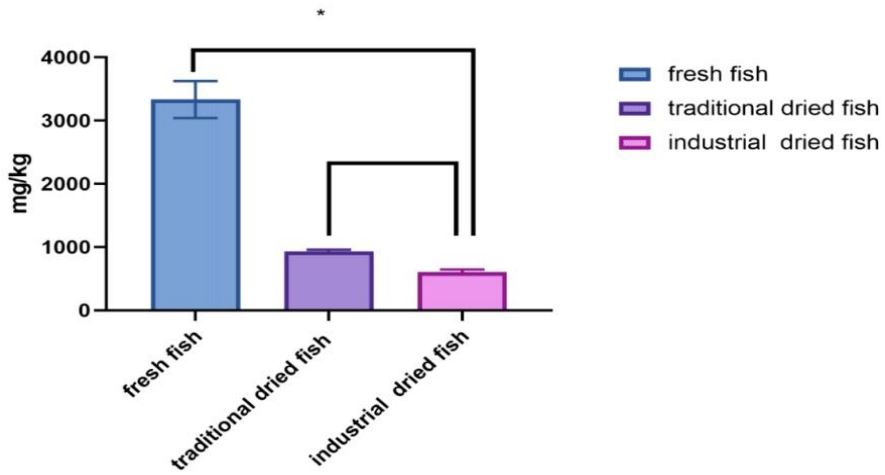

Figure 2. Changes in measured histamine (mg/kg) among fresh, traditional, and industrial dried fish (*P< 0.05).

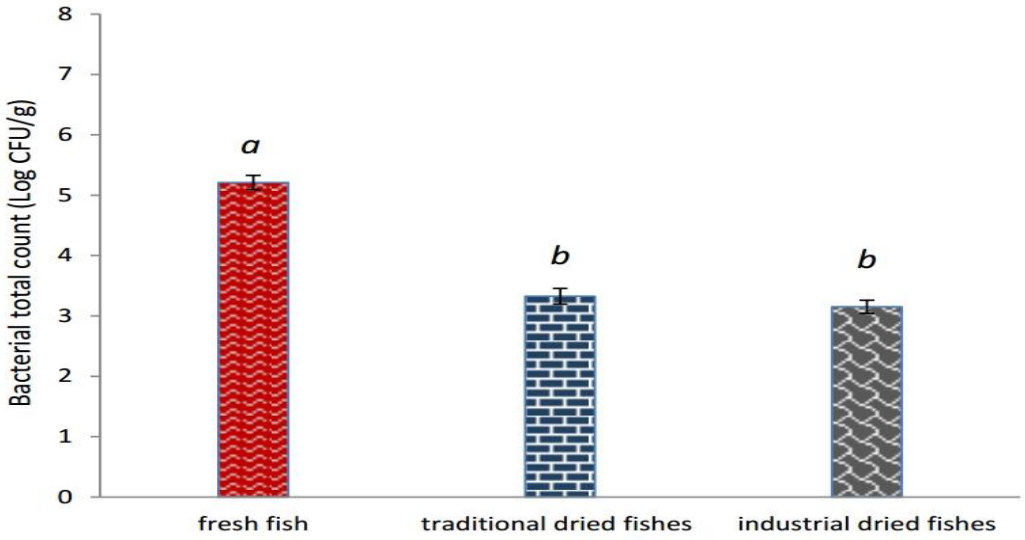

Figure 3. Changes in bacterial total count (Log CFU/g) among fresh, traditional, and industrial dried fish. Non-similar lower case letters indicate significant differences between groups (P<0.05).

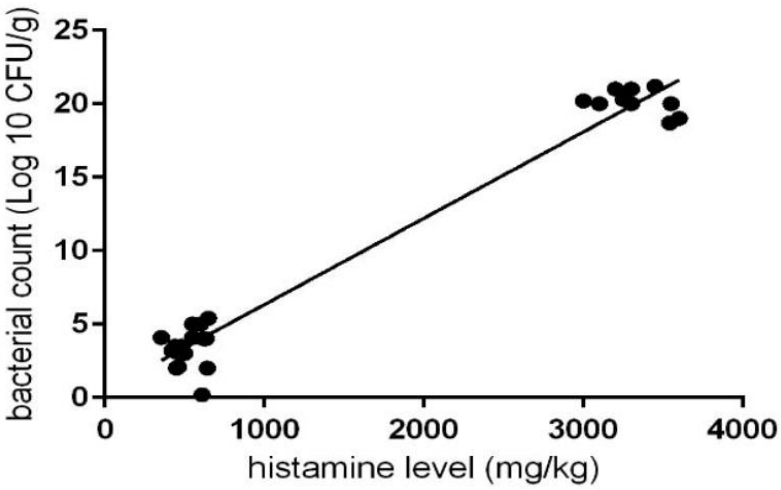

Figure 4. Correlation of bacterial total count and histamine among fresh, traditional, and industrial dried fish (*P> 0.05).

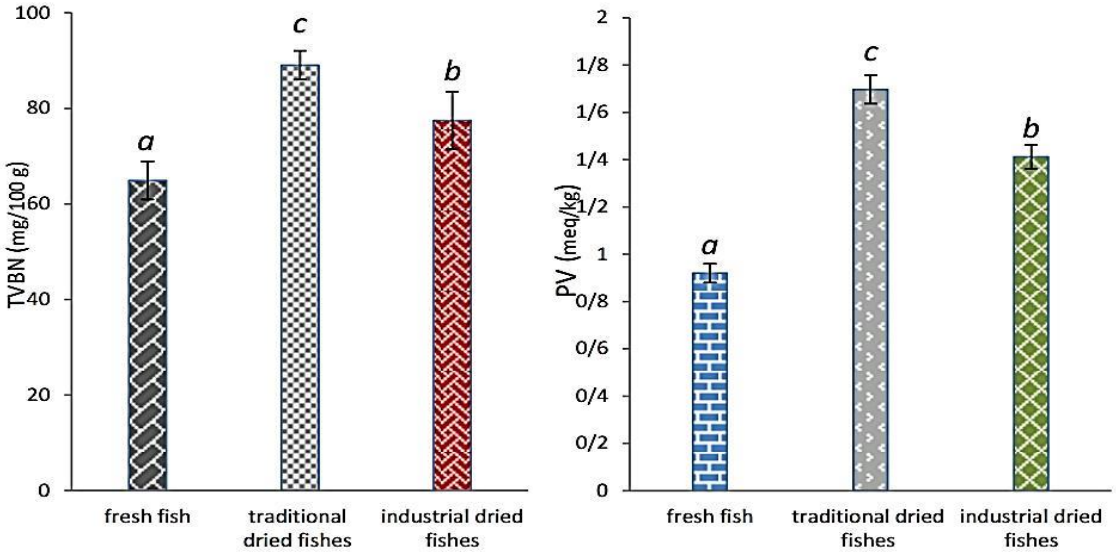

Figure 5. Changes in TVB-N and PV in different groups. Non-similar lower case letters indicate significant differences between groups (P<0.05).

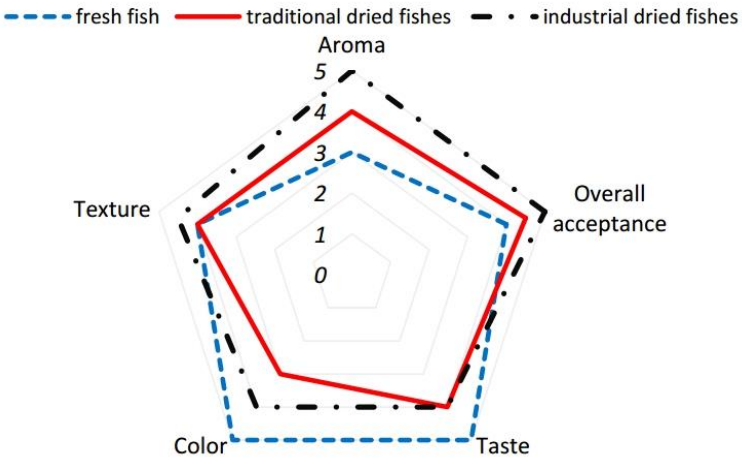

Figure 6. Sensory evaluation results of different groups.

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.The 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 those in fresh fish. This finding suggests 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 deemed 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, and that 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). In a study by Yesudhason et al. (2013), the amount of histamine in fresh, frozen, canned and dried fish samples of scombroid and non-scombroid species from Oman was determined using a high-performance liquid chromatography with a fluorescence detector. The study revealed that histamine was detected in 41.8% of fresh fish samples, 61.0% of

frozen fish samples, 78.9% of canned fish samples, and 91.6% of dried fish samples. This finding is not consistent with the results of the present study, which may be attributable to the fact that the quantity of histamine in dried fish is contingent on various factors, including fishing methodologies, the fishing season, the fish's size, the temperature, and the type of drying process employed (22).Additionally, it was observed that imported dried anchovies exhibited elevated levels of histamine. The study confirms that post-catching and commercialisation practices of seafood are adequate, warranting good quality fish and may not pose a risk of histamine 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 present study observed that the lowest growth was recorded in dried fish processed using the industrial method, thus suggesting 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 found to be related, showing a direct correlation between the two. Rodtonga et al. (2005) investigated the psychrophilic bacteria present in Indian mackerel and found that the highest histamine levels (13.3 mg/200 g) were recorded 40 hours after storage, with an increase in temperature from 35°C resulting in a significant rise in histamine levels (23). Islam et al., (2012) conducted a study that investigated the nutritive and food qualities of traditional, rotary and solar tunnel dried mola (Amblypharyngodon mola). The study concluded that the values of total volatile basic nitrogen (TVB-N) differed among the groups, with the highest values being observed in the traditional samples. N) levels ranging from 10.64 to 20.36 mg/100 g were observed to vary across the various groups. The highest levels were observed in samples dried using traditional methods, a finding that aligns with the results of the present study (24).In a separate study, Tenyang et al. (2020) examined the impact of conventional and industrial drying methods on lipid oxidation and fatty acid composition of two species of freshwater fish. Their findings indicated that both methods significantly accelerated lipid oxidation, as evidenced by an increase in peroxide levels and total oxidation. These observations are in alignment with the results of the present study (25). In the present study, the sensory characteristics, nutritive and food qualities of traditional, rotary and solar tunnel dried mola (Amblypharyngodon mola) products were investigated (24). Organoleptically, the colour of traditional dried mola products was slightly brownish, with the emission of a faint off aroma. In contrast, the color of dried mole fish produced using rotary and solar tunnel dryers exhibited a distinctive shiny colour and whitish to slight brownish hue, though it received lower scores from fresh samples.The texture of traditional dried mola products was somewhat soft, while the texture of rotary and solar tunnel dried products was firm and flexible, accompanied by a characteristic fresh fish-like aroma. The traditionally dried products did not meet expectations, a finding that aligns with the results of the present study. This is of particular importance given the importance of histamine levels in edible fish and the increasing demand for fish as a healthy and common food source. The 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 was responsible for the collection of the samples and the execution of the experimental work. Afshin Akhondzadeh Basti and Asghar Azizian were responsible for the analysis and interpretation of the data. Peyman Mahasti, Shiroorbani, Saeid Tamadoni Jahromi and Mehdi Jabar Zadeh Shiadeh were responsible for the writing and revision of the manuscript.

Ethics
The authors of this study affirm that all procedures were conducted in accordance with the ethical principles established by the ethics committee of the Iranian Veterinary Organization.

Conflict 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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