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Comparative Analysis of Histamine in Fresh and Processed Fish Sold in Jordanian Market

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ABSTRACT

Food poisoning from histamine, a biogenic amine formed due to the decarboxylation of histidine by bacteria in fish and fish products, has become a pivotal concern in food safety. This study measured the concentration of histamine in various fish products available in the Jordanian market, but manufactured in multiple countries, utilizing an Enzyme-Linked Immunosorbent Assay (ELISA). The ELISA kit and the protocol were provided by Veratox for histamine. Approximately 93.69% of the samples tested positive for the presence of histamine, with levels ranging between 0.317 and 230.41 mg/kg. Solely 0.9% of the samples exceeded the maximum permissible level established by the European Union (EU) and only 4.5% of the fish samples were free of histamine. The Principal Component Analysis (PCA) revealed that the type of fish was the most significant source of variability in histamine concentration, explaining 31.2% of the variability. Conversely, the sample weight accounted for the least variability (only 20.2%), implying that it has little or no effect on the concentration of histamine in the fish samples.

Keywords: ELISA, fish samples, food safety, histamine.

INTRODUCTION

Fish are a rich source of essential minerals and proteins, which can meet human nutritional requirements, promoting healthier living (1)(2). It is found that the protein in fish has higher nutritional value than milk, meat, or eggs (3). Additionally, fish provides a good source of omega-3, calcium, phosphorus, iron, and trace minerals. However, the inherent quality protein and polyunsaturated fatty acids in fish make them highly perishable, leading to a reduced shelf life (4). The presence of protein and free amino acids in fish may further decrease freshness due to various enzymatic, biochemical, and microbial activities (5).

Biogenic amines such as histamine, beta-phenylethylamine, tyramine, tryptamine, putrescine, cadaverine, spermine, and spermidine are commonly found in foods and beverages (6). Histamine, in particular, is a biogenic amine produced in fish as a result of the decarboxylation of histidine amino acids by certain bacteria, notably Morganella morganii and Photobacterium phosphoreum (7)(8). This substance can also form through the decarboxylation of amino acids or the amination and transamination of aldehydes and ketones (9).

Exposing fish to elevated temperatures during harvesting and transportation can promote the growth of histidine decarboxylase-producing bacteria, consequently increasing histamine concentration (10). The metabolic activities of

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bacteria convert histidine to histamine in fish, which accumulates in the fish flesh due to a lack of a homeostasis system. To counter this problem, the fish should be iced immediately after they are out of the water (11).

Histamine fish poisoning is often found in species like mackerel, tuna, sardines, sockeye salmon, and amberjack (12). However, the histamine content may change based on factors such as feeding season, fish species, the stage of maturity, and sex (13).

The food-borne chemical intoxication caused by ingesting fish containing high histamine concentrations is termed Histamine Food Poisoning (HFP). This condition can lead to food intoxication and intolerance, and provoke allergic reactions, such as headaches, nausea, acute anaphylaxis, generalized erythema, dyspnea, vomiting, and other discernible symptoms (11, 14, 15). Health effects of histamine may further include mutagenic and carcinogenic actions (16), and it has been observed that normal metabolic activities do not detoxify histamine intoxication (17). The European Commission Regulation (EU) No 1019/2013 on histamine in fishery products (18) stipulates specific criteria for histamine presence. It sets a maximum level of 200 mg/kg and 400 mg/kg of histamine respectively in fresh fish and fish products that have undergone enzymatic maturation or been treated in brine (5, 13). An accurate, fast, and reproducible analytical method with high throughput is required to ascertain the concentration level of histamine in fish and fish products for regulatory compliance and quality control.

The primary analytical methods for determining histamine in fish and fish products include enzyme-linked immunosorbent assay (ELISA) (19), thin layer chromatography (TLC), high-performance liquid chromatography (HPLC) (20), capillary electrophoresis (CE) (4, 5, 21), and gas chromatography-tandem mass spectrometry (GC/MS) (7). Among these, the ELISA method is most commonly utilized for histamine detection because chromatographic methods involve laborious and time-consuming steps for sample pretreatment such as extraction and concentration, along with the necessity of derivatization in gas chromatography (7, 22). This study aims to determine histamine concentration levels in fish samples using ELISA and provide knowledge about the levels of histamine in marketed processed fish in Jordan. This information will improve the understanding of product safety among Jordanians and the health risks associated with the consumption of canned fish.

MATERIALS AND METHODS

Sample Collection

A total of 16 different types of fish samples were collected from 15 different countries, including Belgium, China, Egypt, Indonesia, Italy, Jordan, Morocco, Norway, the Philippines, Thailand, the United Arab Emirates (UAE), and the United States of America (USA). These consisted of nine salmon samples, eight sardines in hot oil samples, seven sardines in hot oil samples, one sardine in olive oil sample, one sardine in vegetable oil sample, one tuna chunk sample, three tuna chunks in hot oil samples, 18 tuna chunks in oil, one tuna chunk in olive oil sample, two tuna chunks in water samples, 16 tuna in hot oil samples, three tuna in olive oil samples, 31 tuna in vegetable oil samples, six tuna in water samples, three tuna with spices samples, and one white tuna in vegetable oil sample. This resulted in an aggregate of 111 samples. Prior to analysis, these samples were stored at temperatures between 11-18°C in a freezer.

Samples preparation and extraction

The sample preparation and extraction process comprised several steps. Firstly, the entire contents of each can, including meat and liquid, were transferred into a blender and homogenized. The blended samples were then stored at a temperature of 2-8°C. Roughly 10 grams of the homogenized sample were weighed into a 125 mL disposable extraction bottle, containing 90 mL of distilled water. This bottle was capped tightly and vigorously shaken for 20 seconds to resuspend the fish tissue completely. It was shaken again for 20 seconds after a 5-

minute interval, and the tissue was then left to settle at the bottom of the bottle. The mixture was centrifuged, and the supernatant was transferred into a clean test tube. Lastly, about 100 µL of the supernatant, or extract, was pipetted into a test tube containing 100 mL of diluent buffer, and the mixture was gently swirled.

ELISA test

All reagents were allowed to reach a temperature range of 18-30°C before use, and the ELISA test was conducted per the manufacturer's instructions.

A red-marked mixing well was set aside for each sample to be tested along with five red-marked wells for controls, and these were placed in the well holder. An equivalent number of antibody-coated wells were also extracted. Unused antibody wells were returned to the foil pack with the desiccant and resealed. All reagents were mixed by swirling the respective bottles prior to use.

The conjugate (100µL) from the blue-labeled bottle was added to each red-marked mixing well. A fresh pipette tip was used to introduce 100µL of the controls and diluted samples into the red-marked mixing wells. Utilizing a 12-channel pipettor, the liquids were mixed in the wells by pipetting up and down three times. 100µL was then transferred to the antibody-coated wells.

The antibody-coated wells were incubated for 10 minutes at room temperature, 18-30°C (64-86°F). This was followed by stirring for the initial 10-20 seconds by sliding the microwell holder back and forth on a flat surface without splashing. The red-marked mixing wells were then discarded. The content of the antibody wells was poured out, each well was filled with diluted wash buffer and emptied three times. After turning the wells upside down, any remaining liquid was blotted onto an absorbent towel.

The required volume of substrate was transferred from the green-labeled bottle into the green-labeled reagent boat. A 12-channel pipettor with new tips was used to pipette 100µL of the substrate into the wells. These were incubated for 10 minutes at room temperature, 18-30°C (64-86°F), and stirred for the first 10-20 seconds. Any remaining substrate was discarded and the reagent boat was filled with water. The same volume of the Red Stop solution from the red-labeled bottle was added to the red-labeled reagent boat. Using the same pipette tips as for substrate dispensation, 100µL of Red Stop was introduced into each well and mixed.

After wiping the bottom of the microwells with a dry cloth or towel, the microwells were read using a 650 nm filter in a microwell reader. Data was analyzed using the Neogen Veratox software, which compared the results to the standard curve to calculate the final readings. It's crucial to ensure no air bubbles form, as this might affect the results. These results should be read within 20 minutes of test completion. Finally, all used materials should be safely disposed of.

Statistical analysis

The concentration of histamine in fish samples was evaluated in triplicate and randomly analyzed. Principal Component Analysis (PCA) was used to determine the relationships (23) between the histamine concentration levels in fish samples, their country of origin, trademark, and sample weight.

Results and Discussion

The concentration of histamine in both imported and locally canned and processed tuna fish products available in Jordanian markets was successfully assessed. Histamine was detected in approximately 93.69% of the samples, ranging between 0.317 and 230.41 mg/kg. In contrast, histamine was not detected in 6.31% (7 samples) of the samples. The histamine-free fish samples primarily originated from Thailand, consisting of five samples (two Sardines in Oil, one Tuna Chunk, one Tuna Chunk in Oil, and one Tuna Chunk in Hot Oil). The remaining two histamine-free samples were Tuna Chunks in Oil from Egypt and Tuna Chunks in Vegetable Oil from Oman. The highest level of histamine, detected at a concentration of 230.41 mg/kg, and the lowest level, found at a concentration of 0.32 mg/kg, were both in 'Tuna in Vegetable Oil' samples from Thailand. Only one sample (0.9%) out of the 111 surpassed the permissible histamine

level of 100 mg/kg.

The highest average histamine concentration of 35.14 mg/kg was found in 'Sardines in Vegetable Oil' (refer to Table 1), whereas the lowest average concentration of 0.72 mg/kg was detected in 'Tuna Chunks in Olive Oil' samples.

Table 1: Average concentration of histamine in fish samples

Fish	Number of samples	Concentration (±SD mg/kg)	Lowest level (mg/kg	Highest level (mg/kg)
Salmon	9	10.34±21.66	0.063	65.15
Sardines in hot oils	8	13.01±15	n.d	44.29
Sardines in oil	7	10.28±12.53	n.d	33.62
Sardines in olive oil	1	7.06±0.00	-	-
Sardines in vegetables	1	35.14±0.00	-	-
Tuna chunks	1	0.00	-	-
Tuna chunks in hot oil	3	0.81±1.30	0.004	2.31
Tuna chunks in oil	18	10.61±12.57	n.d	35.06
Tuna chunks in olive oil	1	0.72±0.00	-	-
Tuna chunks in water	2	3.58±4.43	0.45	6.70
Tuna in hot oil	16	18.02±22.26	n.d	70.14
Tuna in olive oil	3	11.12±10.41	0.47	21.26
Tuna in vegetable oil	31	17.98±41.82	n.d	230.41
Tuna in water	6	22.45±30.42	0.21	77.89
Tuna with spices	3	6.42±5.76	1.57	12.79
White tuna in vegetable oil	1	12.19±0.00	-	-

The results obtained in this study were higher than those reported by Bangieva and colleagues, who recorded a concentration of 13.51 mg/kg in fresh and marine water fish sourced from Bulgarian markets, analyzed using the ELISA method (13). The results were also higher than the 0.211 mg/kg reported by Yusni and colleagues in tuna fish obtained from the Indonesian fishing port (24). However, our levels were lower than the 480.25 mg/kg reported by Diniz and colleagues in commercial seafood collected from Portuguese markets (25).

Our results are comparable to those found by Learoussy and colleagues, who detected histamine levels ranging from 2.74 to 156.60 mg/kg in commonly consumed frozen fish in Mauritania, collected between January and June 2019 (26). Our study agrees with the concentration of histamine ranging from 1.3 to 290 mg/100g found in freshwater fish from Bahir Dar markets in Ethiopia (27). The range is also compatible with the concentration of 17 to 210 mg/100g detected in canned tuna fish sourced from Isfahan, Iran (15).

Table 2: Eigenanalysis of the correlation matrix

Eigenvalue	1.2484	1.0563	0.8866	0.8087
Proportion	0.312	0.264	0.222	0.202
Cumulative	0.312	0.576	0.798	1.00
Variable	PC1	PC2	PC3	PC4
Fish type	0.581	0.379	-0.303	-0.653
Trademark	-0.540	0.402	0.545	0.500
Origin	0.325	-0.706	0.517	-0.357
Weight	-0.513	0.444	0.587	0.442

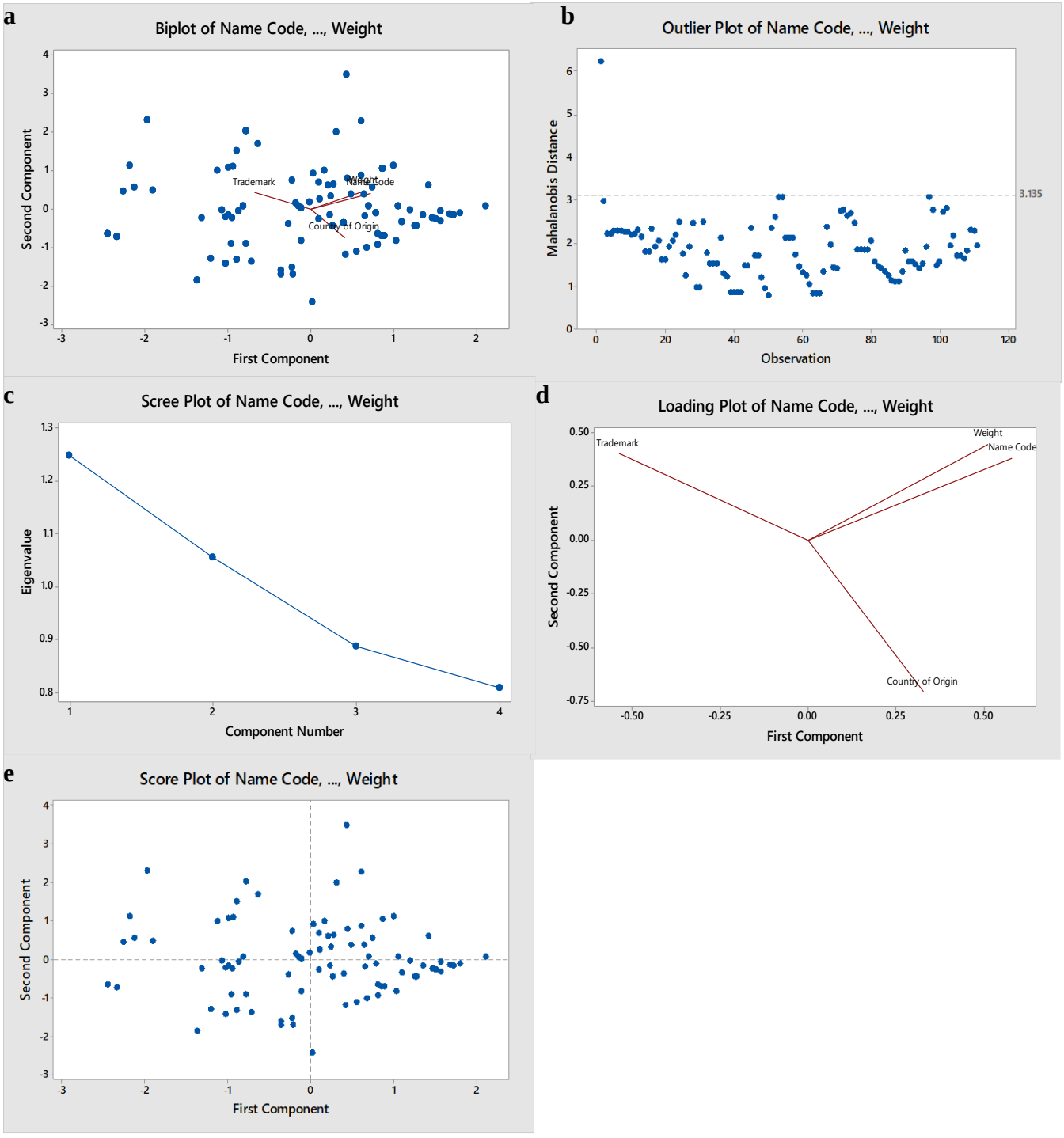


Figure 1: Principal components analysis (PCA) (a) biplot, (b) outlier plot (c) scree plot (d) loading plot and (e) score plot of histamine in fish samples

The Principal Component Analysis, illustrated in Figures a-e and in the Eigen analysis of the correlation matrix (Table 1), showed that the first two principal components have more than 57% impact, with eigenvalues greater than 1 and an accumulative value of 0.567, i.e., 56.70%. The type of fish posed the greatest source of variability and accounted for 31.2% of the variability in the data, making it a crucial component. The least variability was observed in the weight of the samples, displaying a 20.2% contribution to the data variability.

The type of fish had a larger positive association with the weight of the samples, while the trademark had a significant positive association with the country of origin. Conversely, the type of fish demonstrated a larger negative relationship with the trademark. The trademark also exhibited a larger negative association with the country of origin. Meanwhile, the sample weight showed larger negative interactions with the type of fish, the trademark, and the country of manufacture.

CONCLUSION

The histamine concentration identified in various fish was found to be lower than the level deemed safe and tolerable, posing no health risk to consumers, with the exception of one sample that surpassed the permissible limit. The formation of histamine in fish can be curtailed through proper preservation measures, prompt cooling, and efficient refrigeration during processing. These measures can either reduce or completely inhibit microbial activities that can potentially lead to histamine formation. The application of the ELISA kit Veratox for Histamine has proven to be a rapid and accurate means for determining histamine levels in a broad variety of fish species.

Additionally, the use of Principal Component Analysis (PCA) was beneficial in assessing the relationship between histamine concentration and variables such as types of fish, the manufacturer's country of origin, and the weight of different fish products. Future research could explore the use of artificial neural networks (ANN) and partial least squares regression (PLSR) to determine the various environmental factors influencing histamine formation in fish.

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التحليل المقارن للهستامين في الأسماك الطازجة والمعالجة

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ملخص

أصبح التسمم الغذائي بالهستامين، وهو حمض أميني حيوي يتشكل نتيجة نزع كربوكسيل الهيستيدين بواسطة بكتيريا نزع الكربوكسيل في الأسماك والمنتجات السمكية، مشكلة أمنية في مجال سلامة الأغذية. في هذه الدراسة، تم تحديد تركيز الهستامين في منتجات الأسماك المختلفة التي تم الحصول عليها في السوق الأردني ولكن تم تصنيعها في بلدان مختلفة باستخدام مقايسة الامتصاص المناعي المرتبط بالإنزيم (ELISA)، باستخدام مجموعة ELISA وبروتوكول مقدم من Veratox للهستامين. تم الكشف عن الهستامين في حوالي 93.69% من العينات، بحيث تراوحت الكمية بين 0.317 و230.41 ملغم/كغم، مع تجاوز 0.9% فقط من عينات الأسماك الحد الأقصى المسموح به الذي حدده الاتحاد الأوروبي و4.5% فقط من هذه العينات كانت خالية من الهستامين. أظهر تحليل المكونات الرئيسية (PCA) أن المتغير الخاص بنوع الأسماك أدى الى أعلى مصدر للتباين (31.2%)، وهو عامل مهم في تحديد مستوى تركيز الهستامين. ولوحظ أيضا تباين أقل في المتغير الخاص بوزن العينات (20.2%) وبالتالي كان له تأثير ضئيل أو معدوم على تركيز الهستامين في عينات الأسماك.

الكلمات الدالة: مقايسة الامتصاص المناعي المرتبطة بالإنزيم، عينات الأسماك، سلامة الغذاء، الهستامين.

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