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Rapid determination of histamine levels in canned tuna by a novel spectrophotometric method

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ABSTRACT

Histamine is the most common biogenic amine and is responsible for Scombroid fish poisoning. The presence of histamine at high concentrations in foodstuff indicates a public health issue Therefore, to the assurance of safety and quality, the monitoring of histamine concentration in fish and fishery products is urgent. To this aim, histamine content in 30 samples of canned tuna was measured by the UV-Vis spectrophotometry method. Linear regression was gained (R^2 = 0.9905). The range of histamine levels was calculated between 85.04-125.08 mg kg^{-1} with an overall mean of 98.104 ± 5.18 mg kg^{-1}. 40% of the samples were contained more than 100 mg kg^{-1}, the allowable limit declared by Iranian National Standard (INS). However, The histamine amount of all samples were below the limit set by Codex Alimentarius (200 mg kg^{-1}). There was no significant difference between the mean values of histamine in various brands of canned tuna. This spectrophotometric method used in this study can be introduced as a simple, applicable method for rapid monitoring of fish products, which is based on histamine reaction with copper and Alizarin Red S.

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1. Introduction

Biogenic amines (BAs) are nitrogenous, non-volatile lightweight compounds in the form of heterocyclic, alicyclic, and aliphatic organic bases which are found in many types of food (1-3).

Generally, the high concentrations of these compounds have been reported in fish, meat, cheese, some vegetables, beer, and wine, mainly due to the decarboxylation of free amino acids through bacterial activity (4,5).

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The most common biogenic amines include histamine, putrescine, tyramine, cadaverine, and phenylethylamine, which originates from the decarboxylation of histidine, ornithine, tyrosine, lysine, and phenylalanine, respectively (5-7). Histamine [1H-imidazole-4-ethanamine], is a potentially hazardous compound and has historically been a major cause of Scombroid fish poisoning (8). Histamine naturally exists in human body tissues, mainly skin, lungs, and gastrointestinal tract, formed in the Golgi apparatus of basophils and mast cells (9). Histamine receptors mediate some biological and pathological effects, such as allergic reactions, modulation of neurotransmission, inflammation, regulatory immune response, gastrointestinal events, neural disorders, and tumor development (9-12). However, there are several toxicological effects on humans because of high stability during heat processing (3,13).

Scombroid food poisoning also called histamine fish poisoning (HFP), is a foodborne illness related to the consumption of fish containing high levels of histamine that continues to be a significant public health issue (14, 15). Typically symptoms of HFP include rash, diarrhea, vomiting, flushing, sweating, and headache, and the severity of these symptoms differs with the amount of histamine consumed and the individual's sensitivity (16), which can be a mild illness or in some sensitive cases cause death (17). The U.S. Food and Drug Administration (US FDA) has regulated 50 mg kg^{-1} as a maximum concentration for histamine in seafood products, which no adverse effects on this concentration have been observed on health (18, 19). According to the national standards of Iran, the limit of histamine has been set to <100 mg kg^{-1} (7). Moreover, various analytical techniques are used for the concentration of histamine, including Gas chromatography (GC), High-Performance Liquid Chromatography (HPLC), Thin Layer Chromatography (TLC), fluorometric assay, colorimetric assay, ultrasensitive flow injection electrochemical analysis, immunoassay methods, and spectrophotometry (6,13,20,21). Because the use of complicated methods such as chromatography is costly and time-consuming, thus the use of spectrophotometry is especially suitable for rapid monitoring of finished products in factories before releasing them to the markets, with good applicability and feasibility. Alizarin Red S easily forms complex colors with organic and metallic substances such as copper (22).

Furthermore, the concentration of histamine can be determined spectrophotometrically at a wavelength of 600 nm, based on its reaction with copper and Alizarin Red S. This study aimed to apply a novel spectrophotometric method for the rapid screening of histamine levels in commercial canned tuna samples from Tehran's market, Iran.

2. Materials and Methods

2.1. Samples collection

Samples of canned tuna (n=30) were purchased from different supermarkets located in Tehran in July 2020. All samples were transferred to the laboratory and were stored at room temperatures before analysis. All samples were analyzed in duplicate.

2.2. Reagent and buffer preparation

Reagents $CuSO_4.6H_2O$ and Alizarin Red S were prepared as follows: 0.39 g $CuSO_4.6H_2O$ and 0.1 g of Alizarin Red S were weighed and dissolved in distilled water, respectively, and moved into a 100 ml volumetric flask to a final volume of 100 ml. The buffer solution was made using a phosphate buffer solution containing sodium hydrogen phosphate dehydrate (2M) and sodium dihydrogen phosphate dehydrates (2M). Finally, buffers that were previously prepared were used to achieve the required buffer with pH 7.

0.25 ml of the previously prepared Alizarin red S solution was transferred to a 10 ml volumetric flask and then 1 ml of Cu (II) solution and 1 ml buffer solution (pH 7) were added. Then methanol was poured to a final volume of 10 milliliters.

2.3. Calibration curve

To prepare the standard histamine solution, 0.1 g of histamine was weighed and then dissolved in 20 ml methanol. The prepared solution was moved to a 100 ml volumetric flask and to achieve the mark, methanol was added. Concentrations of 100, 150, 250, 500, and 1000 mg kg^{-1} were prepared from standard histamine solution to draw the calibration curve.

2.4. Sample preparation and extraction

The whole canned fish sample was thoroughly homogenized with a laboratory blender, then 5 g of each canned fish was weighed and transferred into a 50 ml centrifuge tube, and 20 ml of methanol was added. In the next step, it was shaken vigorously for 5 min and then homogenized for 10 min using an ultrasonic bath (TAT, China). Afterward, the sample was centrifuged (Premium 20000R, PIT Co., Iran) for 15 min with 4500 rpm. About 1.25 ml of the supernatant was picked up and transferred to another tube, then 1 ml of $CuSO_4.6H_2O$, 0.25 ml of Alizarin Red S and 1 ml of buffer solution were added and then passed through filter paper (Whatman No. 42). The spectrophotometer was set on zero by the blank sample (methanol) before placing the real samples every time and then the absorption wavelength was performed. The best optimized response time was observed at 10 min after sample preparation. The absorbance of this complex solution was measured by a UV-Vis spectrophotometer instrument (Hach DR5000, USA) at a wavelength of 600 nm, which is wavelength of the complex compound of histamine, Alizarin Red S and Cu (II) (20).

2.5. Statistical Analysis

Statistical differences between brands were evaluated using the student's T-test by SPSS software (SPSS Inc., USA) version 22.0 for windows. The Kolmogorov-Smirnov test was used to determine whether the distribution is normal or not. The data values are expressed as the mean ± standard deviation and range. Linear regression analysis was examined with Excel 2013 (Microsoft, Redmond, WA). According to the statistical facts,a significant difference was concluded at a level of $p \leq 0.05$. Figures were plotted with Prism 8 (Graph Pad, San Diego, CA).

3. Results and discussion

In the present study, the concentration of histamine at 30 samples of canned tuna was analyzed. As shown in the figure 1, the linear regression equation was $y = 0.0013x - 0.1076$, and $R^2 = 0.9905$. The limit of detection, the limit of quantification, and recovery percentage

were obtained about 11.5 mg kg^{-1}, 32.8 mg kg^{-1}, and 89%, respectively. The range of histamine levels was calculated between 85.04 - 125.08 mg kg^{-1}. The highest and lowest mean amount of histamine belongs to the brand D (119.31 ± 8.16 mg kg^{-1}) and brand N (85.04 ± 0.92 mg kg^{-1}), respectively. The overall mean of total samples was obtained at 98.104 ± 5.18 mg kg^{-1}. None of the canned tuna samples were contained above 200 mg kg^{-1} of histamine, the maximum limit suggested by Codex Alimentarius. 40% (n=12) of the samples were contained more than 100 mg kg^{-1}, the allowable limit declared by INS, which is not acceptable for consumption. There was no significant difference between the mean values of histamine in various brands of canned tuna. Histamine was detected in all samples, illustrated in figure 2. It is noteworthy that all samples were produced in the summer of 2018. During analysis, most samples were at the end of their expiratory date. That is probably why histamine was found in all samples. The most widespread investigations in fishery products among the BAs are on histamine, due to its importance in causing scombroid fish poisoning, and it is the only amine with a legal limit for human consumption (3). The presence of high levels of histamine can indicate the poor quality of raw materials, spoilage, improper storage conditions, and other poor manufacturing processes in food products especially seafood (6, 23). Histamine concentrations above 200 and often above 500 ppm in illness-causing fish have been reported due to fish consumption (13). According to the codex guidelines, the maximum allowable limit of histamine concentration is 200 mg kg^{-1}, while the INS has established this limit below 100 mg kg^{-1} (7).

According to previous studies conducted in Iran, histamine has been detected in most samples by various measurement methods. In a recent study by Peivasteh-roudsari et al. (2020), the amount of histamine in different types of canned fish including Tuna, Sardine, Kilka, and Mackerel was determined by HPLC-UV. The findings which were in line with our study showed that 46.6% of the samples contained histamine and 18.3% of the samples exceeded the FDA limit. Also, histamine content was reported in the range of 0 to 88 mg kg^{-1}, with an overall mean ± SD of 17.36 ± 15.44 mg kg^{-1} for all samples (7). Sadeghi et al. (2019) evaluated histamine concentration in canned tuna and fresh fish with an enzyme-linked immunosorbent assay (ELISA). they found histamine in the range of 2.14 ± 0.17 to 21.69 ± 0.11 mg 100 g^{-1}, which all samples were lower than the allowable limit suggested by FDA in contrast with the present study (24).

In another study that was fulfilled by the ELISA method, Hosseini et al. (2007) reported histamine concentration in 44.3% of samples more than the permitted limit by FDA (50 mg kg^{-1}), and 23.8% of the samples have shown to contain histamine above 150 mg kg^{-1}. Also, a mean histamine concentration of about 68.7 ± 28.46 mg kg^{-1} was obtained (25). Zarei et al. (2011) detected histamine by HPLC-UV in the range of 0.12 - 648.20 mg kg^{-1} at 57% of the samples, and 25% of them were more than the recommended limit by FDA (26). Histamine concentration of the canned tuna samples that evaluated by Synergy HT Multimode Microplate Reader, from 4-236 mg kg^{-1} has been reported by Zarei et al. (2010) which 18.9% of these samples exceeded the FDA limit (27).

Akbari-Adergani et al. (2010) investigated the histamine concentration in canned tuna fish samples by ELISA and FFT-StCV methods. Histamine concentrations were found in the range of 0.1 to 259 mg kg^{-1}, with the mean±SD of 59.6 ± 22.4 mg kg^{-1} for all samples. 36.6% of samples were above 50 mg kg^{-1} the allowable limit suggested by FDA and 13.3% more than 150 mg kg^{-1}, which was in agreement with the current study (28). Kamkar et al. (2008) in their study on the determination of histamine content in canned tuna samples by ELISA, reported 82.81% of the samples contained histamine which was 31% of these samples more than the permitted limit by FDA. The highest contamination belonged to the summer samples, which is compatible with our results (29).

According to Emirhan et al. (2017), the mean ± SD of histamine concentrations in canned tuna fish was determined 10.97 ± 9.86 mg kg^{-1} in fish samples using ELISA, and only 10.0% of samples were positive for histamine that all samples were lower than the allowable limit suggested by Turkish Food Codex values (200 - 400 mg kg^{-1}) (30).

Tsai et al. (2005) investigated the amount of histamine in different types of canned fish including Tuna, Mackerel, Bonito following the occurrence of food-borne poisoning in Taiwan was determined by HPLC. They have reported a considerable amount of 153.9 mg $100g^{-1}$ of histamine in canned mackerel that is exceeded the FDA limit (31). The present study data about histamine content is supported by previous studies mentioned above.

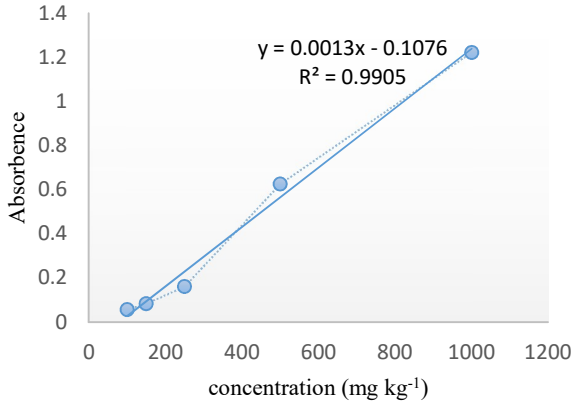

Figure 1. Calibration curve of histamine

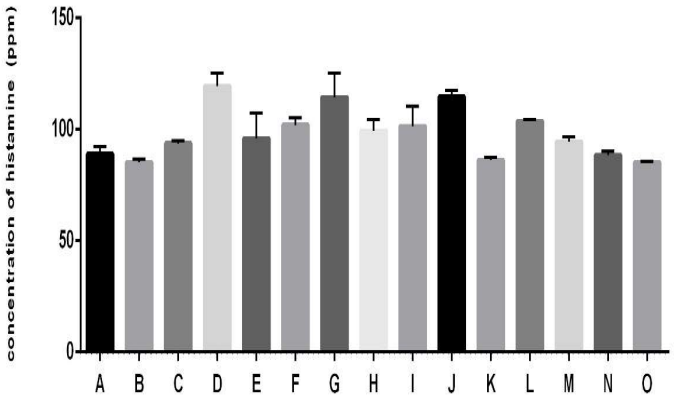

Figure 2. Comparison of histamine concentrations in various brands of canned tuna

4. Conclusion

Given the global importance of food safety and the adverse reaction at high levels of histamine on health, monitoring histamine content in seafood such as canned fish applying a simple and fast method seems very necessary all over the world. The mean of histamine values was 98.104±5.18 mg kg^{-1} with a range of 85.04-125.08 mg kg^{-1}. Regarding the INS maximum limit (100 mg kg^{-1}), 40% of the samples have shown

exceeding values of histamine. However, the histamine amount of all samples were below the limit set by Codex Alimentarius (200 mg kg^{-1}). The samples with the highest levels of histamine belonged to the samples produced in summer, probably meaning the improper storage of raw fish or contamination during transportation.

The spectrophotometric method used in this study, which was based on histamine reaction with Cu (II) and Alizarin red S, can be used in factories as a fast, simple, cost-effective, and applicable method for rapid and initial monitoring of final products, although it may not be very accurate compared to the cost and time-consuming methods such as HPLC which possess high accuracy and precision.

Conflict of interest

Authors declare that they do not have conflict of interest.

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