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Determination of histamine in canned tuna fish available in Tehran market by ELISA method

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ABSTRACT

Histamine is one of the well-known biogenic amines and responsible for causing allergic reactions. The presence of biogenic amines in the foodstuff is harmful, if it enters in a large amount to blood. In sea-food products, due to lack of proper storage at appropriate temperatures (freezing), histamine may be formed and will remain in the product, since it is already dry and heat resistance. Hazard of histamine consumption and average amount of canned fish consumed worldwide makes histamine measurement in canned fish very important. In this study 56 samples from 22 different brands were assessed and Enzyme-Linked Immunosorbent Assay (ELISA) was used by spectrophotometry for histamine detection. Our study showed that histamine levels in canned fish available in Tehran market, though it is high (5.75±5.98 mg/100 g tuna), but is not in a hazardous state ($p<0.01$). Our research showed that lowest and highest histamine concentration were 2.14 ±0.17 and 21.69±0.11 mg/100 g of fish respectively. It also indicates that medium does not affect the histamine content. There were no significant differences in the samples of fish and tuna fish for histamine. The amount of histamine in the tuna was below the standard limit (<50 mg histamine/100 g). Further studies should be carried out to investigate the presence of histamine in various fish and other sea-food.

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1. Introduction

Histamine, or beta-amino imidazole, is a hydrophy drophilic molecule and one of the most important biogenic amines. This compound, as a natural anti-nutritional agent, can cause food poisoning in humans (1). Tuna fish has high economic and nutritional value and is an important fishing target (3). Scombridae fishes travel long distances without rest, and to prevent fatigue, a natural solution for them is a high concentration Histidine amino acid in their muscles. Fish immune system halts after death and normal flora starts to multiply. Bacteria metabolism turns Histidine to Histamine and due to lack of homeostasis system, Histamine condenses is fish flesh. The only way to prevent this process is icing it immediately after catching. Histamine is not sensitive to heat and dryness, so if icing is not done immediately, any product made by that fish, like canned fish, will be contaminated with Histamine. Consuming Histamine may lead to allergic reactions from rhinorrhea to anaphylactic shock (4). Danger of Histamine consumption and average amount of canned fish consumed worldwide makes Histamine measurement in canned fish very important. Sanchez-Guerreroet al in 1997, a patient was admitted in hospital suffering from dyspnea, vomiting, diarrhea, generalized erythema and pruritus. Symptoms appeared 20

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minutes after consuming canned tuna fish. Patient seemed to be experiencing acute anaphylaxis caused by allergy. He was treated with epinephrine, fluids, oxygen, steroids and antihistamines and symptoms disappeared in 15 to 17 h (5). Such incidents are known as "Scombroid Poisoning" and considered as an allergic-like reaction caused by consumption of tuna or any other member of Scombridae family. Scombridae family members travel long distances in a relatively high speed. As a natural solution to prevent fatigue, their muscles are rich in Histidine amino acid which is metabolized to Histamine and Histamine-like by bacterial micro-organisms after the fish dies. To achieve this goal, fishes must be buried in ice powder the moment they're caught. If proper icing, preserving and preparation conditions are not met, histidine metabolism toward histamine begins right after fish is dead and its immune and homeostasis systems are halted. Different countries or locations may have different standards and guidelines on maximum allowed histamine in fish and canned fish but in most of them less than 5 milligrams histamine in 100 grams of fish or canned fish is assumed safe. Public Health Laboratory Service in the UK has published a guideline on fish products histamine toxicity risk that would be the base of further conclusions.

Symptoms of scombroid poisoning include flushing, rash, urticarial, palpitations, headache, dizziness, sweating, burning of mouth and throat, abdominal cramps, nausea, vomiting, diarrhea, bronchospasm, respiratory distress and vasodilatory shock. Though very rare, death by scombroid poisoning has been reported once (6,7) and more lethal incidents may happen in future. The purpose of this study was to measure histamine contents of 56 canned tuna fish and fresh fish samples produced from 22 manufactures, analyzed by ELISA.

2. Materials and methods

2.1. Sample collection

As a sample to begin with, 56 cans of tuna from 22 different manufacturers bought randomly of random stores in Tehran, Iran. Also, 3 types of fresh fish were gathered in order to compare histamine content of canned fishes with fresh ones. Canned and fresh transported to lab and stored in -20°C freezer.

2.2. Measuring histamine

Histamine concentration measured by competitive ELISA (RIDASCREEN®Histamin,96 well, R-Biopharm , Darmstadt, Germany). To prepare the sample, 10 g of tuna was weighed and flesh and medium was homogenized using lab blender (Features of the device), then 1 g of the blend was moved to a falcon test tube and 9ml de-ionized water was poured on it. Test tube was centrifuged with 2500 RPM for 5 min in room temperature (Features of the device). After that, 3 phases was formed: oily, aqueous and solid, from top to bottom respectively. Oily phased was discarded and 1ml of aquatic phase was transferred to another test tube and 9ml de-ionized water was added. As a final dilution 200µl of the test tube contents was added to 9.8ml de-ionized water in the third tube. Finally, using the kit, the sample was a cylated and A cylated histamine was binded to the antibody coated on the well surface after adding the sample. Then washed off the excessive sample. Enzyme conjugated antibody was added, and washed. After incubation time, provided stopper solution was added as the final step read sample absorbance in 450 nm with using BioTeK®Microplate reader (PMT 49984, U.S.A).

2.3. Method accuracy and Precision

To define accuracy of the method, a standard sample in the kit with concentration of 15mg was measured using the same method used for samples preparation. The concentration was measured 14.62 mg, so accuracy of the method is equal to 97.47%.

Precision can be inspected in 2 sub-category; Repeatability and reproducibility. To define them, a sample was selected and histamine content was measured three times in the first day (Repeatability) and also, once in next two days (Reproducibility). Data of the first day were 2.73, 2.97 and 2.80 and second and third day were 2.16 and 2.54 respectively; so repeatability is equal to 0.04 and reproducibility is equal to 0.11.

2.4. Statistical analysis

Data obtained from ELISA reader were transformed to descriptive data using computer assisted statistics application SPSS (Version 16.0.0, IBM Corporation, Armonk, New York, United States). Independent Sample T-test, One-Sample T-test and One-way ANOVA were performed and differences assumed significant at *p-value* less than 0.05.

3. Results

Results of histamine measurement are summarized in Table and visualized in Figure 1Figure

2 shows the concentration of histamine in various environments such as oil, brine and other liquids (olive oil, canola, and vegetables). Figure 3 shows the concentration of histamine in various brands of tuna fish. Canned fish from different manufacturers was also compared to determine if it affects the histamine content. Each brand name was labeled with alphabet letters, and for brand names with more than one flavor, the average of all products was used.

Table 1. Histamine Concentration

scale	Canned Tuna (mg/100gTuna)	Fresh Fish (mg/100gFish)	p-value
Samples Count	56	3	
Average	5.78±6.14	5.14±0.85	> 0.05
Minimum	2.14±0.17	4.19±0.04	> 0.05
Maximum	21.69±0.11	5.81±0.08	> 0.05

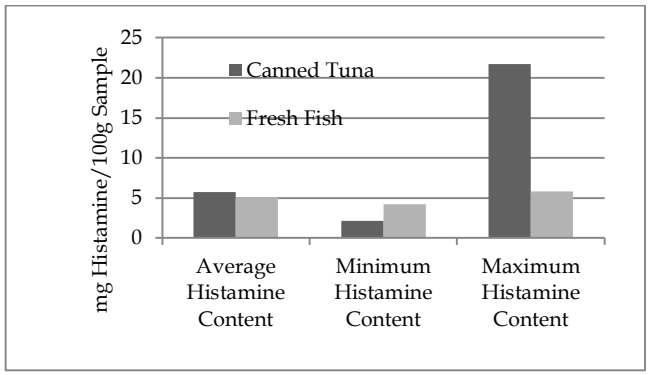

Figure 1. Fresh Fish vs Canned Tuna

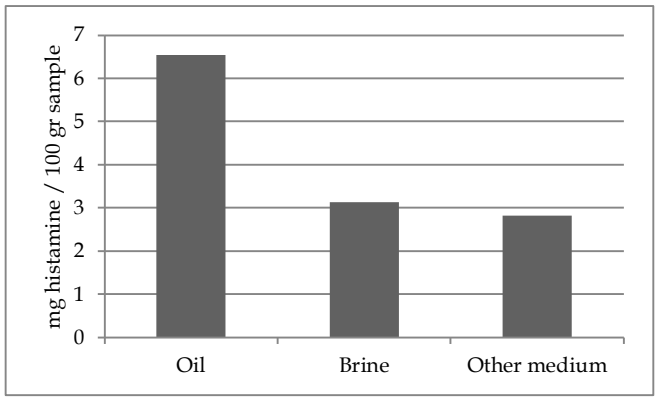

Figure 2. Histamine Level Comparison According to Medium

Canned tunas are labeled with expiry date of 2 years after production. We divided samples in according to the time passed from production in groups of 6 months differences: first 6 months, second 6 months and finally, third 6 months of production date. Canned tunas with more than 18 months passed from their production date are recalled by producing company in order to prevent expired tuna consumption. Figure 4 shows the effect of the novelty

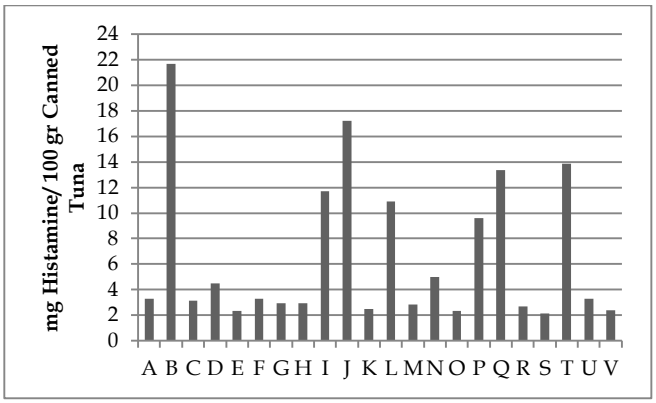

Figure 3. The level of histamine in canned fish of different brands in Tehran
Brand name: A, B, C

of tuna fish on histamine content. Cans within their 6, 12 and 18 months of production was measured 2.33 ±0.21, 3.45±0.53 and 13.15±7.50 mg/100 g of tuna respectively.

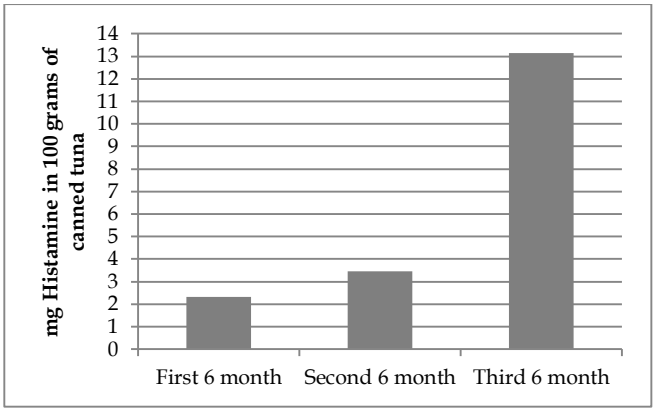

Figure 4. Mean Histamine Concentration According to Production Time

4. Discussion

Histamine concentration in canned tuna is over 5 mg /100 g of fish that places the average safety marker of the Tehran market in "Mishandled and Possibly Toxic" level. Histamine concentration is higher in canned tuna than the fresh fish, but statistical suggest that this difference in not significant ($p > 0.05$) (Table 1). Histamine concentration varies depending on medium that fills the can and measured 6.54±4.73 mg, 3.13±0.80 mg and 2.82±0.95 mg per 100 g of fish in brine, oil and other mediums respectively. Figure 2 compares the histamine content of cans with different mediums though the difference was not statistically significant ($p > 0.05$). Therefore, if the oil, the salts, and other environments contained within the fish are not contaminated with histamine, it does not affect the

amount of the present in the fish. Figure 3 shows the result and difference in histamine concentration was statistically significant ($p < 0.05$). "B" had the highest, while "S" measured as the lowest histamine concentration among samples. According to Fig. 4the difference was statistically considered significant (p-value < 0.05). In attention to FDA data, safety limits of histamine content of food determined as<5 mg/100 g as safe, 5-20 mg/100 g presumably toxic, 20-100 mg/100 g likely toxic and> 100 mg/100 g as toxic levels. So, FDA introduces the limit of 50 mg/100 g (8). As you can see from the comparison, histamine production can't be reduced to zero, because it's a natural by-product of a natural metabolism. In most of the cases histamine content was below standard limits, and it seems that best way to prevent toxicity is regular testing of the products at the manufacturing site and releasing each batch only after it's tested Safe. Regardless of whether using fresh or canned fish and no matter what medium is used to produce canned Tuna, consumption of some species may carry the risk of scombroid toxicity incidents. Since histamine doesn't change color, smell or taste of food, the only way to make sure the fish being consumed by people is safe is regular sampling and testing of fish and its products and setting regulations for proper storing and preparing such food-stuffs. As time passes, risk of corrosion of can and increase bacteria metabolism increases. Findings suggest cans with more than a year passed from their production show increased concentration of histamine and it's recommended to use tuna Tons of tuna fish produced recently. Several similar works have been done in the field of fish and fish products all around the world; for example, in 1996, a study on 27 samples showed histamine was traceable in all of the samples but none of them exceeded European limits (9). According to a study by Aminiet al., in 2018 on the histamine of fish tuna, it became during time intervals (0, 5, 10, 15 and 20 days) the histamine content in all samples significantly increased ($p<0.05$) (10). Sadeghi et al (2015) reported that the maximum and minimum histamine levels were 12.6,4.6 ppm, respectively. In frozen samples, the highest and lowest histamine were 2.6, 0.75 ppm, respectively (11). Köse et al (2003) added some histamine to fish fillets and found that cooking caused a significant decrease in histamine but could not completely eliminate it. They found that rapid freezing of fish immediately after harvesting and storage at low temperature significantly reduced histamine formation in products (12). Ozlem et al. (2008) examined the histamine content of different fish species and found that all species were higher than a standard value (13). Kung et al showed tuna sandwich products were also identified as prolific histamine formers (14,15). Lee et al (2013) found E. aero genes and R. isolated from the suspected raw striped marlin fillets were identified as prolific histamine formers, able to produce >129 ppm of histamine in TSBH medium (16). In this study, there was less histamine content in tuna products in Iran than in foreign tuna products which can be attributed to fish species, fish storage temperature and environmental health status for fish processing.

5. Conclusion

Average concentration of histamine in canned tuna fishes available in I.R. Iran Market, is slightly higher than the level considered safe but such amount seems to be tolerable for healthy consumers without excessive sensitivity to histamine, although, efforts should be done to improve storing and preparing methods to decrease histamine production to minimum. The constant control of the histamine presence in food rich and proteins should be introduced, because of the possibility of histamine development in such foodstuffs, detrimental to human health. Since the"screening" method for quantitative determination of histamine is easy to perform, the control of histamine presence should be legally regulated for the protection of human health.

Conflict of interests

The authors have no conflict of interest.

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