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## Histamine content of marine fillet fish in Kermanshah

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ABSTRACT: Histamine is an important cause of poisoning that classified as biogenic amines. Some enteric bacteria that could produce histamine isolated from fish. This study aimed to determine histamine levels in marine fillet fish in Kermanshah, to evaluate risk of fish poisoning in this region. The study was carried out on four fish species, Otolithes ruber, Scomberomorous commerson, Liza aurata, Lutjanus johni. Samples were taken from Kermanshah fish markets during autumn 2012-13. To measure Histamine level a competitive ELISA was employed using a Lab System plate reader and Neogen Histamine kit. All data were analyzed by using ANOVA, the LSD test and SPSS 18. Results showed that all of specimens contained detectable value of histamine. Maximum and minimum value of histamine in fresh fillet was recorded in O. ruber (12.6 ppm) and L. aurata (4.6ppm), separately. In frozen samples the highest value was recorded in S. commerson (2.6 ppm) and the lowest value was seen in L. johni (0.75 ppm). In the present study, histamine has also been found in frozen samples. This indicates poor storage conditions especially for temperature during storage and distribution. Therefore, accurate and continuous monitoring during storage of fish is necessary.

Keywords: Histamine, Marine fillet fish, ELISA, Kermanshah.

#### INTRODUCTION

Histamine is an important cause of poisoning that classified as biogenic amines (1, 2). It is a foodborne chemical intoxication caused by eating spoiled, or bacterial contaminated fish. Fish is healthy eating when fresh, and might gradually contaminated and become toxic while have a normal appearance and aroma (3). Scombroid fish or fish associated with histamine fish poisoning (HFP) contain high levels of free Histidine (100 mg/100 g fish muscle) in tissue and are often implicated in scombroid poisoning (1, 2). Histamine is a normally occurring element in mammalian physiology. It is restricted in mast cells and basophiles, and its biological properties are commonly. It might unconstrained in great amounts in the sequence of allergic and re-actions. Histamine effects by binding to receptors in the gastrointestinal, haematological /immunological systems and the skin (4). Scombroid poisoning have many severe clinical symptoms such as rash, urticaria, nausea, vomiting, diarrhea, edema, localized inflammation, flushing, tingling abdominal cramps, headache, palpitation, severe respiratory distress and itching of the skin (1). Histamine formed by the decarboxylation of histidine and exogenous decarboxylases released from the bacteria which are related to the seafood (5). Some enteric bacteria such as Enterobacter aerogenes, Enterobacter cloacae, Proteus vulgaris, Serratia fonticola, Serratia liquefaciens, Raoultella (Klebsiella) planticola, Acinetobacter lowffi. Plesiomonas shigelloides. Pseudomonas putida. Pseudomonas fluorescens and Aeromonas spp which may produce histamine isolated from fish (6-9). Histamine being formed in high temperature after capture and its accumulation is affected by the combination of time and temperature (10). This study was done to determine histamine levels in marine fillet fish including Otolithes ruber, Scomberomorous commerson, Liza aurata and Lutjanus johni in Kermanshah, Iran to evaluate risk of fish poisoning in this region.

#### **MATERIAL AND METHODS**

The study was carried out on four fish species, O. ruber, S. commerson, L. aurata and L. johni. All samples were taken from Kermanshah fish markets during autumn 2012-13. The analysis was carried out in Food Microbiology Laboratory of Kermanshah University of Medical Sciences. Competitive ELISA was employed to measure histamine using a Lab System plate reader and Neogen Histamine kit (11). The assay was performed according to the manufacturer's recommendation. A limit of detection of this test for fresh fish and canned fish is 5 ppb. After the sample preparation, histamine was quantitatively converted to Nacylhistamine, using an acylation reagent. Free acylated histamine and bound histamine compete for the antibody binding sites in the competitive ELISA. After washing, the secondary peroxidase-conjugated antibodies (enzyme conjugate) are added. These antibodies bind to the antibody-histamine complex. Unbound antigen then removed by washing. Substrate (urea peroxide) and chromogen (Tetramethyl-Benzidine) are added into wells of the micro-titration plate and then incubated. During incubation, the bound enzyme conjugate converts a colourless chromogen into blue product and blue color changes into yellow after addition of stop solution. After the substrate reaction, the optical density is measured at 450 nm on the ELISA plate reader (Lab System Multiscan, Finland). The amount of complexes bound to the plate and the optical density are inversely proportional to the histamine concentration of the sample (11). All data are expressed as means ± standard deviation (SD). The differences in the parameters were tested for significance using a one-way analysis of variance (ANOVA) and the LSD (least-significant difference) test (P<0.05). Statistics analysis was done with SPSS 18.

#### **RESULTS**

Our results showed that all of specimens contained detectable value of histamine. Maximum and minimum value of histamine in fresh fillet was recorded in O. ruber (12.6 ppm) and L. aurata (4.6 ppm). In frozen fish, the highest value was recorded in S. commerson (2.6 ppm) and the lowest value was seen in L. johni (0.75 ppm). As seen in fig. 1 histamine content was higher in fresh samples in compare to frozen. In all samples histamine content was lower than standard level except for fresh samples of L. aurata that was higher than standard values.

Table 1. Histamine contamination level means in fresh fillet marine fish

| Mean(ppm) ± SD | NO. sample | Type of fish |
|----------------|------------|--------------|
| 7.3 ± 2.1      | 20         | L. johni     |
| $4.6 \pm 0.7$  | 20         | L. aurata    |
| 12.6 ± 1.5     | 20         | O. ruber     |
| 10.2 ± 1.4     | 20         | S. commerson |

Table 2. Histamine contamination level means in freeze fillet marine fish

| 1 | Mean(ppm) ± SD | NO. sample | Type of fish |
|---|----------------|------------|--------------|
| ( | 0.75 ± 0.09    | 20         | L. johni     |
| ( | 0.8 ± 0.054    | 20         | L. aurata    |
| 2 | 2.4 ± 0.82     | 20         | O. ruber     |
| 2 | 2.6 ± 0.9      | 20         | S. commerson |

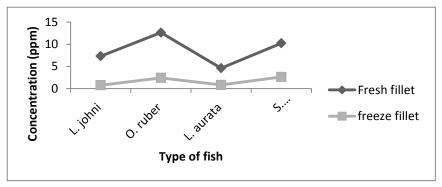


Figure 1. Comparison of histamine contamination level in fresh and freeze fillet marine fish

#### **DISCUSSION**

In the present study, histamine has also been found in frozen samples. This indicates poor storage conditions especially adjusting temperature during storage and distribution. Therefore it's possible to stop the formation of histamine in fish if they were kept in -1°C after fishing. The highest value was recorded in S. commerson and the lowest value was seen in L. johni. Japanese investigator found histamine poisoning for first time in 1950s. Based on epidemiologic findings, it was the most relevant food born disease in that period. Some reports of histamine poisoning from tuna fish and mackerel had been reported from fish (12). Histamine content of fresh fish was lower than 2ppm and fish that contain more than this value may cause some level of poisoning. Fish considered spoiling when histamine content is 20-50 ppm and values more than 50 ppm considered as an active dose that is none consumable (13). Histamine content of fish had been reported in different studies. Kasha & Noriens (1988) found Histamine contents in S. commerson lower than Mackerel (14). Gajewska et al (1991) found Histamine contents were in fish from 0 to 8 mg/100g in Poland (15). During 1988-1991 histamine level of fresh fish was lower than standard in mackerel and tuna samples randomly in Italy (16). Chamberlin and Makan in 3 species of tuna fish found that histamine level in fish kept in low temperatures was lower than standard and was increased by increasing temperature (17, 18). Histamine is potentially toxic; so many countries have set control values of histamine in fish and other marine foods very carefully. Özlem et al (2004) determined histamine content in fillet of anchovy, horse mackerel rainbow trout barbell and found that in all species it was higher than a standard value that was higher compare to our finding (19). In anchovy samples collected during 2006, histamine content in 58% of samples was higher than standard value (20). Takahashi et al (2003) evaluated histamine content in three marine species, mackerel, bonito and anchovy and found that it was lower than could induce toxicity (21). Histamine is heat-stable toxin however, cooking and preparation methods such as freezing, canning and making smoke could decrease its amount in the foods (6). Köse et al (2003) studied the histamine amount in mackerel fillets and found that cooking reduces the amount of histamine but cannot eradicate it (22). Rapid fish cooling after harvest and storing at low temperature could decrease histamine formation in products (22, 23). As noted above, histamine does not destruct by thermal processes. Collected fish samples contained histamine that was higher than standard values. It could be concluded that storage of fish had not been done correctly, but in frozen samples it was lower than fresh fish. Low humidity and temperature during storage could prevent spreading of histamine. Therefore, careful and continuous monitoring during storage of fish is necessary.

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