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Evaluation of Histamine in Canned Tuna Fish Post Market Samples in Iran Using ELISA

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In the present study, histamine contents of 88 canned tuna fish products from several part of Iran during 2006-2007 were analyzed by using ELISA method. The results showed that 44 % of samples had higher histamine contents than FDA caution level (> 50 ppm). Furthermore the samples from the producers of southern provinces of Iran contained higher amount of histamine than the other in northern parts. The present study also revealed that the histamine amounts depend on production date and increase by closing to expiration date of samples. The results suggested that hygienic quality of canned tuna should be improved by implementing more strict time/temperature controls during commercial processing (cold chain supply).

Key Words: Scombroid, Histamine, Chromogen, ELISA, Tuna fish.

INTRODUCTION

Histamine fish poisoning or scombroid[1] is an acute allergy-like food poisoning occurs mostly due to eating fish containing high levels of histamine and is one of the most frequent intoxications related to sea food consumption[2,3]. Decarboxylative conversion of histidine to histamine during fermentation of enterobacteriaceae[4], lactic acid bacteria[5] and photobacteria[6] on scombroid fish such as tuna is the source of histamine accumulation in susceptible fish. Based on European Union Legislations and FDA regulations histamine levels in sea food must not exceed 100-200 ppm and 500 ppm, respectively but FDA set to keep the caution levels at 50 ppm[7]. Histamine is resistant to thermal processes (freezing, cooking, canning, *etc.*) and the only way to prevent its accumulation in fish is storing the fish below 4 °C[8]. Rapid removal of viscera and washing the fish can significantly reduce the population of histamine producing bacteria and can be regarded as another effective approach for reducing the histamine levels in fish[9]. Several methods have been proposed for

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histamine analysis in foodstuff. Fluorescence measurement of histamine after derivatization with *o*-phthaldehyde is one of these methods with overall suitability but the method is time consuming and needs pre-analysis clean-up[10]. High-performance liquid chromatography (HPLC) can be applied for analysis of histamine and some other biogenic amines in fish in a quantitative manner but this method relies on advanced operating techniques[11,12]. Enzymatic assays using histamine oxidase[13] or histamine dehydrogenase[14] are simple and rapid methods for histamine analysis but cross reaction with putrescine and tyramine make some difficulties for selectivity of the method due to co-eluted samples with histamine. Enzyme-linked immunosorbent assay (ELISA) kits have been recently used for histamine determination in cheese and some other foods[15,16]. Due to good sensitivity and simplicity of the analytical application by these kits we used enzyme immunoassay method for determining histamine contents in 88 canned tuna fish brands produced in different geographic locations of Iran. The aim of the present study is to evaluate the safety of different brands of canned tuna fish in respect to histamine content for retail consumers.

EXPERIMENTAL

Two sets of 88 different brands of canned tuna fish (with same sample batch number) without any physical damage before expiration date were collected from retail market and transferred to Food and Drug Control Laboratories Research Centre for analysis. One set of samples was kept as control. Number of samples (sample size) was calculated based on a previous study[17]. The Ridascreen® histamine (Art. No. R1604) is a competitive enzyme immunoassay was purchased from R-biopharm AG, Dermstadt, Germany. All the reagents required the enzyme immunoassay-including standards and controls- are contained in the test kit. In this method after sample preparation, histamine is derivatized by an acylation reagent into N-acyl histamine. In a competitive ELISA, free acylated histamine and bounded histamine compete for the antibody binding sites. After washing, secondary antibodies labeled with peroxidase (enzyme conjugate) are added. These antibodies bind to the antibody histamine complexes. Any unbound enzyme conjugated antibody is then removed in a washing step. Enzyme substrate (urea peroxide) and chromogen (tetramethylbenzidine) are added to the wells and incubated. Bound enzyme conjugate converts the colourless chromogen into a blue product. The addition of the stop solution leads to a colour change from blue to yellow and the measurement is made spectrophotometrically at 450 nm.

Sample preparation: A 100-200 g portion of each sample after discarding the oil were cut up in a blender at high speed in 5 min, 10 ± 0.1 g of the ground samples were homogenized in distilled water (90 mL) and 1 mL of the homogenized samples were transferred to a falcon tube and centrifuged at 2500 g for 5 min at room temperature. The lipid layer was removed. The supernatant (20 µL) was diluted with 10 mL distilled water.

Test procedure for acylation: 100 µL of each standard solution, control or prepared sample were added to separate wells of acylation plate. The acylation reagent (25 µL) and buffer (200 µL) were added to each well. Then the plate was mixed gently by shaking manually and incubated for 15 min at room temperature.

Test procedure for ELISA: To the acylated standard solution, control or prepared samples (25 µL), 100 µL of the anti-histamine antibody was added and mixed gently by shaking manually for 40 min at room temperature. The liquid poured out of wells. All the wells were filled with 250 µL of washing buffer and poured out the liquid again. This procedure was repeated two more time. The substrate/chromogen solution (100 µL) was added to each well and mixed gently and incubated for 15 min at room temperature in the dark. The stop solution (100 µL) was added to each well and mixed gently by shaking the plate manually and measured the absorbance at 450 nm against an air blank in spectrophotometer (Bio-rad ELX 50) within 10 min after addition of stop solution. Each sample was extracted and analyzed in triplicate. All standards, controls and samples were run in duplicate. The detection limit of the method for histamine was 2.5 ppm. A calibration curve for histamine determination was obtained using standard solutions (0, 0.5, 1.5, 5, 15, 50 ppb) in the kit. Furthermore the recovery from the spiked tuna fish samples was obtained and the correction factor was used for results in the base of recovery. The data was evaluated by using ANOVA multivariate in SPSS statistical software.

RESULTS AND DISCUSSION

The histamine contents of canned tuna fish samples in this study were in the range of 2.5-214 ppm. Histamine contents in 44.3 % of the samples were higher than 50 ppm while this value was higher than 150 ppm in 23.8 % of the samples. The mean histamine contents and standard deviation of samples were measured as 68.7 ± 28.5 ppm (Table-1).

TABLE-1
FREQUENCY OF HISTAMINE CONTENTS IN CANNED TUNA FISH SAMPLES

Histamine content	Number of samples (n = 88)	Frequency (%)
< 50 ppm*	49	55.7
15-150 ppm**	18	20.5
> 150 ppm***	21	23.8

*Lower than allowed amount. **Between allowed amount and three fold of allowed amount.
***Upper than three fold of allowed amount.

As shown in Table-2 histamine contents of analyzed samples (60 %) with no later 6 months were below the FDA caution level, while 18 % of samples with similar production status showed histamine contents with only more than three times of that limit. The samples with histamine content lower than 50 ppm and production dates shorter than 6 months were 68.2 % of total. The results of present study indicated a significant correlation between the histamine contents of the samples based on production and analysis interval ($p < 0.05$).

TABLE-2
HISTAMINE CONTENTS OF SAMPLES REGARDING TO THE PRODUCTION-ANALYSIS INTERVAL

Histamine content	Production-analysis interval (months)		
	Less than 6 months	6-12 months	More than 12 months
< 50 ppm	32** (65.3%)*	11 (22.4%)	6 (12.3%)
15-150 ppm	11 (61.1%)	2 (11.1%)	5 (27.8%)
> 150 ppm	6 (28.6%)	–	15 (71.4 %)

*Frequency percentage, ** Sample number.

The present data also revealed the influence of geographic site of production facilities on the histamine contents of the canned tuna fish. Production sites were classified based on location in northern, central and southern provinces of Iran. As shown in Fig. 1 the samples produced in southern provinces had the highest histamine content (93.6 ± 24.5 ppm). This was significantly different from the histamine content of canned tuna fish brands produced in southern and northern provinces ($p < 0.05$). It should be mentioned that there was no significant difference between the samples produced in central and northern provinces in term of quantity regarding to the histamine contents.

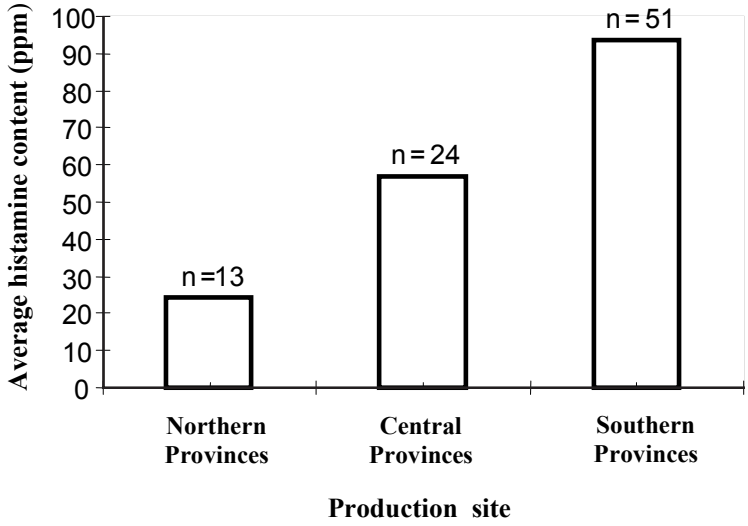

Fig. 1. Histamine contents of analyzed canned tuna fish samples based on geographic production sites

Histamine content of tuna fish or its processed food product is a valuable factor for evaluating the quality of products[18]. Scombroid is a world wide food poisoning but its prevalence has been reported higher in Japan, England and the United states that could be attributed to either higher consumption of scombroid fishes or more efficient tracking and reporting food poisoning systems in these countries[19]. The

results of this study revealed that 55.7 and 44.3 % of canned tuna fish samples studied in this report contained histamine levels lower and higher than 50 ppm (caution level regulated by FDA), respectively. These results were in accordance with a previous report determined by a chemical histamine analysis method and detection limit 5 ppm[17]. In that study 41.25 % of samples contained histamine levels above the mentioned limit. However the range of histamine contents of samples in the present study (2.5-214.3 ppm) were higher than the previous study (10-178 ppm). It could be due to application of different analytical methods. Upon to another trial in Australia during 1996 the histamine content of 51 % of domestic canned fish products and 16 % of imported products were higher than approval limits[20]. The results of present study is indicative for higher average histamine content in analyzed canned tuna fish samples with longer intervals between production and their analysis dates. 65.3 % of all the samples in which histamine content was lower than the regulated limit had been produced not more than 6 months far from the analysis date, while 71.4 % of samples in which histamine content was more than 3 times of the regulated limit had been produced later than 1 year before their analysis. It has been previously shown that decarboxylative enzymes responsible for conversion of histidine to histamine could remain active due to mal-practice and temperature abuse in the process of storage, transport and canning of tuna fish[8]. Based on the results of present study it is evident that geographic site of production facilities is an important factor in the histamine content of analyzed samples. While histamine contents of analyzed canned produced in northern and central provinces of Iran were 44.9± 19 ppm (lower than caution level of FDA), this value in samples produced in southern provinces of Iran was determined 93.6 ± 24.5 ppm. Keeping in mind that most of the production facilities located in southern parts of Iran have lower distance to shore. Then it is probable that low quality tuna fishes often transfers to these-closer sites while the tuna fishes with better quality freezes instantly after fishing and preserves in good conditions supplied to production sites in central and northern provinces. An alternative reason for explanation of this difference could be due to higher average temperature of southern part of Iran in comparison to northern and central parts that could affect the activity of bacteria responsible for production of histamine during the defrosting cooking/sterilization lag time and increase the histamine content of final product. Based on the results of a previous studies exposure of tuna fish samples even for a short period to temperatures ≥ 30 °C every day and for longer than 3 days could significantly increase the histamine concentration of the sample and prone the consumers to fish histamine toxicity.

It is recommended that all production facilities not only act based on GMP (good manufacturing practices) regulations but also strictly decrease the sample exposure times to ambient temperature. These sites should be kept cool enough (< 20 °C). Inspection of tuna fish at production sites and exclusion of any sample with high histamine content from production cycle would be beneficial to increase the overall quality of the product.

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