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Biogenic Amines Content of Canned Tuna Fish Marketed in Iran

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Abstract: Canned tuna fish are frequently and largely produced and consumed in Iran and also exported. Therefore, their biogenic amines content should be of some concern to human health. In the present study, the levels of histamine, putrescine, tyramine and spermidine in 40 samples of canned tuna fish were determined. Histamine, as the major chemical hazard of seafood products, was detected in 57.5 % of the samples tested. It was in the range of 0.12-648.20 mg/kg. 25 % of the samples contained more than 50 mg histamine/kg fish, the allowable limit suggested by US FDA. Tyramine, as the major mutagen precursor, was detected in 55 % of the samples tested. The overall mean of tyramine in canned tuna samples was 0.7 mg/kg. This level of contamination is far below the allowable level of tyramine in foods. Putrescine was detected in all the samples tested in the range of 0.29-52.83 mg/kg. However, spermidine was only detected in 32.5 % of the samples analyzed with the overall mean of 1.37 mg/kg. Considering high levels of histamine in canned tuna samples, quality control programs in the tuna-canning industries must recommend removing contaminated fish and improving the quality of canned tuna fish.

Key words: Biogenic amine · Canned tuna · Histamine · Putrescine · Spermidine · Tyramine

INTRODUCTION

Biogenic amines are organic bases with aliphatic, aromatic or heterocyclic structures that can be found in several foods, in which they are mainly produced by microbial decarboxylation of amino acids, with the exception of physiological polyamines [1]. The most important biogenic amines; histamine, tyramine, tryptamine, putrescine and cadaverine, are formed from free amino acids, namely histidine, tyrosine, tryptophan, ornithine and lysine, respectively. Spermidine and spermine arise from putrescine. These free amino acids either occur as such in foods, or may be liberated through proteolysis. Microbial strains with high proteolytic enzyme activity also potentially increase the risk for biogenic amine formation in food systems, by increasing the availability of free amino acids. In addition to the availability of precursors (i.e. amino acids), biogenic amines accumulation in foods requires the presence of microorganisms with amino acid decarboxylases and favorable conditions for their growth and decarboxylation activity [2].

Biogenic amines are of public health importance due to the risk of food intoxication and serve as chemical indicators of food spoilage, namely fish, meat and meat products [3]. Low levels of biogenic amines in food are not considered a serious risk. However, when consumed in excessive amounts, they may cause distinctive pharmacological, physiological and toxic effects. Typical symptoms of biogenic amine intoxication in human can be nausea, respiratory distress, hot flushes, sweating, heart palpitation, headache, bright red rash, oral burning and hypertension as well as hypotension [4]. Apart from the toxic effects described above, biogenic amines can play a role in other kinds of processes harmful to human health. Various biogenic amines when subjected to heat, can give rise to the formation of secondary amines and in the presence of

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nitrites, these can generate nitrosamines, chemical agents considered to possess major carcinogenic properties [5]. Moreover, high levels of biogenic amines were reported in breast and colon cancer cells [6]. The quantity of biogenic amines is also to be considered as a marker of the level of microbiological contamination in food [7]. For these reasons, it is important to monitor biogenic amines level in food.

Canned tuna fish are frequently and largely produced and consumed in Iran and also exported. Five species of scombroid fish, including Skipjack tuna (*Katsuwonus pelamis*), Yellowfin tuna (*Thunnus albacares*), Long tail tuna (*Thunnus tonggol*), Frigate tuna (*Auxis thazard*) and Kawakawa tuna (*Euthynnus affinis*) are generally used in the canning industries in Iran [8]. We previously reported that 18.9 % of the canned tuna samples marketed in Iran contain more than 50 mg histamine/kg fish, the allowable limit suggested by US FDA [8]. However, to the best of our knowledge, there is no information on biogenic amines content of canned tuna fish marketed in Iran. Therefore, the present study was carried out to generate information on biogenic amines concentration of canned tuna fish. In this study, the levels of histamine, putrescine, tyramine and spermidine in samples of canned tuna fish were reported.

MATERIALS AND METHODS

Sampling: Canned tuna samples of different factories were purchased from popular supermarkets in Iran. After opening each can, oil was drained off and the meat was ground in a food blender with stainless steel cutters for 3 min. Samples were then taken for determination of biogenic amines concentrations.

Reagents: All reagents were of analytical grade. Standard biogenic amines, including histamine dihydrochloride, putrescine dihydrochloride, spermidine trihydrochloride and tyramine hydrochloride were obtained from Sigma (St. Louis, MO, USA). Methanol, trichloroacetic acid (TCA), benzoyl chloride, NaOH and diethyl ether were purchased from Merck (Darmstadt, Germany).

Determination of Biogenic Amines: Forty samples (10 g, each) were homogenized with 50 ml of 6% trichloroacetic acid (TCA) for 3 min. The homogenates were filtered through Whatmann No. 2 filter paper. The filtrates were placed in volumetric flasks and TCA was added to bring to a final volume of 100 ml. The benzoyl derivatives of the standard biogenic amines solutions and the tuna fish extracts were prepared according to Hwang *et al.* [9]. Briefly, one ml of 2 M sodium hydroxide and 10 µl of benzoyl chloride were added sequentially to 2 ml of standard biogenic amines solutions or the tuna fish extracts. The resulting solution was vortex mixed and allowed to stand at 30°C for 45 min. Benzoylation was stopped by adding 2 ml of saturated NaCl solution and the mixed solution was extracted with 3 ml of diethyl ether. After centrifugation, the upper organic layer was transferred to a test tube and evaporated to dryness. The residue was dissolved in 500 µl of methanol and 20 µl aliquots were used for HPLC analysis. A HPLC unit (Shimadzu 10A-VP) equipped with a UV-Vis detector (set at 254 nm) and class-VP software was employed. A C18RS 250 by 4.5 mm column was used, with 70:30 (v/v) methanol: water as the mobile phase at the flow rate of 1 ml/min.

RESULTS AND DISCUSSION

Fish are rich sources of high-quality proteins, essential vitamins and healthful polyunsaturated fatty acids. The high content of proteins, on the other hand, represents a risk in the decomposition processes. The disintegration of proteins yields peptides and amino acids, which are susceptible to further decay, resulting in biogenic amines that can be widely distributed in proteinaceous foods especially fish [10]. In the present study, the concentration of four biogenic amines; histamine, putrescine, tyramine and spermidine in canned tuna fish was determined (Table 1).

Histamine is identified by the Food and Drug Administration (FDA) as the major chemical hazard of seafood products. It is the causative agent of scombroid poisoning and is formed by time/temperature abuse of fish muscle [11]. Quality loss and histamine accumulation

Table 1: Biogenic amines content (mg/kg) of canned tuna fish

	% of samples with	Minimun	Maximum	Mean[a]	S.E.	Median[a]
Histamine	57.5	0.12	648.20	64.61	24.17	2.37
Putrescine	100.0	0.29	52.83	10.03	1.69	6.12
Tyramine	55.0	0.46	9.70	0.70	0.24	0.46
Spermidine	32.5	0.12	32.41	1.37	0.80	0.00

[a] Mean and median values were calculated by using 0 mg/kg for samples with not detectable levels of biogenic amines

often occur after frozen fish are thawed and kept for long periods of time at room temperature before canning. Since histamine is heat resistant, it can remain intact in canned or other processed fish products [12]. The FDA has established 50 mg/kg guideline for histamine and intensified its inspection efforts. Seafood products containing above this level of histamine must not be used for human consumption and are subjected to recalls [13]. The European Union (EU) has established regulations for histamine levels, such that they should be below 100 mg/kg in raw fish and below 200 mg/kg in salted fish for species belonging to the *Scombridae* and *Clupeidae* families [14]. According to Parente *et al.* [15], histamine intake range within 8-40 mg, 40-100 mg and higher than 100 mg may cause slight, intermediate and intensive poisoning, respectively.

In the present study, histamine was detected in 57.5 % of the samples tested. It was in the range of 0.12-648.20 mg/kg with the overall mean of 64.61 mg/kg. 10 % of the samples contained more than 100 mg histamine/kg fish, 15 % of the samples contained 50-100 mg/kg and 25 % of the samples contained more than 50 mg/kg, the allowable limit suggested by US FDA [13]. According to Tsai *et al.* [16], only 4.2 % of the canned fish samples in Taiwan contained more than 50 mg/kg histamine and most of the tested canned fish products had low levels of histamine. The same results were reported by Yen and Hsieh [17]. Lopez-Sabater *et al.* [12] detected 1.35 mg/kg histamine in canned herring, 30.9 mg/kg in canned tuna, 16.3 mg/kg in canned sardine and 28.3 mg/kg in canned mackerel. While, Ababouch *et al*. [18] noted that 7 % of canned tuna contain histamine at levels greater than 500 mg/kg, 5 % of canned tuna samples in this study contain more than 500 mg histamine/kg. Histamine levels higher than 2000 mg/kg have been reported in canned sardine, mackerel and tuna [19].

It seems that the use of poor quality fish as raw material for canning and/or defective handling techniques of fish during processing are the main reasons of this high percentage of unacceptable canned tuna samples. It is well documented that histamine production is associated with the growth of bacteria that possess the enzyme histidine decarboxylase. In fish, several histamine-producing bacteria have been implicated as primary contributors to histamine formation. They are *Morganella morganii*, *Klebsiella pneumoniae*, *Enterobacter aerogenes*, *Proteus vulgaris*, *Hafnia alvei*, *Citrobacter freundii*, *Serratia spp*. and *Escherichia coli* [19-21]. These bacteria are capable of producing hazardous amounts of histamine in a very short period of time when the fish are kept at elevated temperatures. According to Lopez-Sabater *et al.* [12], an increase in bacterial population occurs while the frozen tuna are being defrosted and handled for processing. Furthermore, contamination of tuna with histamine-producing bacteria can also occur from the environment or the equipment in the processing plants. The knife used for cutting the tuna and peeling the skin, if not properly cleaned, can cause cross contamination.

Tyramine has been identified as the major mutagen precursor [22]. In our study, tyramine was detected in 55 % of the samples tested. It was in the range of 0.46-9.7 mg/kg with the overall mean of 0.7 mg/kg. This level of contamination is far below the allowable level of tyramine in foods. Nout [23] pointed out that maximum allowable level of tyramine in foods should be in the range of 100-800 mg/kg.

Putrescine and spermidine have no adverse health effect, but they may react with nitrite to form carcinogenic nitrosoamines and also can be proposed as indicators of spoilage [24]. Furthermore, high levels of putrescine and spermidine have been recognized as potentiators of histamine or tyramine toxicity, but no recommendations about levels have been suggested. In our investigation, putrescine was detected in all the samples tested; in the range of 0.29-52.83 mg/kg. However, spermidine was only detected in 32.5 % of the samples analyzed with the overall mean of 1.37 mg/kg.

In conclusion, high levels of histamine in 25 % of the samples can pose a health risk to consumers. Therefore, quality control programs in the tuna-canning industries must recommend removing contaminated fish and improving the quality of canned tuna fish.

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