

DETERMINATION OF HISTAMINE LEVELS IN CANNED TUNA FISH

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Abstract

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The presence of biogenic amines in foods is a chemical indication of food spoilage. Therefore, monitoring of biogenic amines levels in food and food products is important. In this study, total number of 80 canned tuna fish samples of four different brands (A, B, C, D) collected from Ankara local markets, Turkey were analyzed for histamine and pH value. Extraction and determination of histamine in all samples were made by enzyme-linked immunosorbent assay (ELISA) procedure. Positive samples were reanalyzed by capillary zone electrophoresis technique with diode array detection (CZE-DAD). The mean levels (\pm S.D) of histamine were found to be 10.97 ± 9.86 mg kg⁻¹ in fish samples by using ELISA. The levels of four samples were determined by capillary zone electrophoresis technique. However, other four positive samples were below limit of quantitation value (5.18 mg kg⁻¹). Our data revealed that eight (10.0%) of canned tuna fish samples were positive for histamine. The mean levels in positive samples were within the Turkish Food Codex (TFC) values (200-400 mg kg⁻¹). This study indicated that some canned fish sold in Ankara, contains histamine, however, these levels were within allowed limits. The pH values of samples were within the Turkish Standard Institute (TSI) values (4.0-6.9). The mean pH values (\pm SE) of the samples for the A, B, C and D brands were determined to be 5.89 ± 0.02 , 5.86 ± 0.01 , 5.83 ± 0.02 and 5.82 ± 0.02 , respectively.

Key words: Canned tuna fish; biogenic amine; histamine; capillary zone electrophoresis; enzyme-linked immunosorbent assay

Introduction

Histamine is the causative agent of scombroid poisoning, a foodborne chemical intoxication (Visciano et al., 2007). Such potentially harmful compounds are found in some fish species, where high levels of free histidine decarboxylation products exist (WHO, 2011; Prester, 2011). Scombridae and Scomberesocidae families with high content of free histidine in their muscles have been found to be commonly involved in scombroid poisoning (Visciano et al., 2007). Histamine [2-(4-Imidazolyl)-ethylamine] is a biogenic amine produced by some species of bacteria, such as *Morganella morganii* and *Proteus* spp., occurring to various degrees in many foods (Maintz et al., 2006; WHO, 2011). Histamine-rich foods include red wine and beer, fish, cheese, sausage, salami, and many other favorite foods. Histamine is produced by microbiological action in the food processing (Maintz et al., 2006). Therefore, it is found in substantial levels in many fermented foodstuffs and beverages. The consumption of his-

tamine rich foods causes diarrhea, headache, rhinoconjunctival symptoms, asthma, hypotension, arrhythmia, urticaria, pruritus, and flushing in histamine-sensitive patients (Maintz and Novak, 2007). In general, if the human is exposed too little histamine levels, histamine are not absorbed efficiently by the gastrointestinal tract. On the other hand, food-borne histamine may permeate through the intestinal barrier. In this case, histamine intoxication may occur if sufficient levels of histamine enter the blood (Taylor, 1986). Exposure to high levels of histamine containing foodstuffs cause histamine poisoning (Valls et al., 1999). Histamine fish poisoning (or scombroid poisoning) is of an important public health and safety concern (Silva et al., 2011). The symptoms of histamine poisoning are nausea, vomiting, diarrhea, oral burning sensation, itching and rash (Macan et al., 2000). Histamine has biologically strong effects such as directly stimulation of the heart causing extra vascular smooth muscle contraction or relaxation. It stimulates both sensory and motor neurons and controls gastric secretion. Consequently, histamine fish

poisoning have various signs or symptoms (Numanoğlu et al., 2008). Symptoms are diminished by antihistamines or may be reduced by a histamine free diet (Maintz and Novak, 2007).

The presence of biogenic amines in foods is a chemical indication of food spoilage. Therefore, monitoring of biogenic amines levels in food and food products is important (Zarei et al., 2011). In the Turkish Food Codex (TFC), the levels of histamine are regulated with limit values as 200 mg kg⁻¹ (m) and 400 mg kg⁻¹ (M) in canned fishery products (TFC, 2011). According to the European Union Regulation (EC) No 2073/2005 histamine limit values for brined fishery products are 200 mg kg⁻¹ (m) and 400 mg kg⁻¹ (M) (EC, 2005).

The aim of this study was to determine levels of histamine in the eighty canned tuna fish samples consumed in Ankara Region to evaluate whether these levels were within the TFC values or not. For achieving this, various approaches such as CZE and ELISA have been applied.

Material and Methods

Samples

Eighty canned fish samples of oil and light consistency with different serial numbers were collected from different brands (A, B, C, D) in Ankara supermarkets, Turkey. These samples were collected before their expiration date without any physical damage.

Chemicals

The standard histamine was obtained from Sigma-Aldrich (Steinheim, Germany). All the reagents were of analytical grade. Methanol (CH₃OH), NaH₂PO₄·2H₂O, Phosphoric acid (H₃PO₄), hydrochloric acid (HCl) and sodium hydroxide (NaOH) were obtained from Merck Chemical Co. (Darmstadt, Germany). Deionized water was used throughout the experiments (Barnstead International, D11901 USA).

Analytical analysis

Measurement of pH value of samples

The pH values of samples were determined by pH meter (Hanna pH 211, Romania) according to Marco et al. (2006).

ELISA analysis of histamine

Histamine was determined by an ELISA method using the Ridascreen Histamine/ELISA kit (R-Biopharm AG, Darmstadt, Germany) (Anonymous 2012). Sample preparations were done according to the instructions of the Ridascreen kit. Briefly, each of the canned fish sample was homogenized using Turrax disperser (Turrax T18 Basic IKA, Germany) and 1 g of the homogenized sample was transferred to centrifuge tubes, 9 mL distilled water was added to the samples and

mixed well by vortexing (Firlabo SA, France). Subsequently, these were centrifuged at 2500 g for 5 min at room temperature (Jouan MR 1822, France) and afterwards lipid layer was removed. 1 ml of supernatant was transferred into other centrifuge tube, 9 mL distilled water was added and mixed well by vortexing. Then, 200 µL of this solution was diluted with distilled water. 100 µL of the standard solutions and prepared samples and controls were added to 96 wells in the acylation plate, respectively. 25 µL of the acylation reagent and 200 µL of the acylation buffer were added to each acylation well and mixed before incubation for 15 min at room temperature. 25 µL of acylated standard solutions, controls and prepared samples were used for the ELISA procedure. The absorbance was measured at 450 nm in ELISA Reader (Versamax Tunable Microplate Reader, BN02636). Concentrations of histamines were calculated through the guidelines of the Ridascreen kit (Anonymous, 2012). Ridasoft Win PC-Software for the evaluation of data was used. The detection limit was reported as 2.5 mg kg⁻¹, being the lowest value for canned fish in the test kit.

Capillary zone electrophoretic analysis of histamine

Capillary zone electrophoresis conditions

The histamine levels in the fish samples were determined by the method of Numanoğlu et al. (2008). Analyses were performed by a capillary zone electrophoresis apparatus (Agilent HP 3D CE Technologies, USA) which consisted of a diode array detector (DAD) set at 214 nm. The capillary utilized had an effective length of 50 cm, and 50 µm of I.D. (Agilent Technologies, USA). Peak area was used for the simultaneous determination of histamine in canned tuna fish samples. The separation voltage was 25 kV. The separation buffer was prepared with NaH₂PO₄·2H₂O and Na₂HPO₄·2H₂O and adjusted to pH value with H₃PO₄ (pH=2.5).

For conditioning, a new capillary column was rinsed with 1.0 M NaOH, deionized water and 1.0 M phosphoric acid. The capillary was rinsed with 0.1 M phosphoric acid (8 min) and running buffer (12 min) at the beginning of each run (Numanoğlu et al., 2008).

Stock solution containing 1000 µg/mL histamine was prepared by dissolving 10 mg of histamine in 10 mL of 0.1M HCl. Standard solution was prepared by diluting the stock solution with 0.1 M HCl (5-200 µg mL⁻¹). All solutions were used after filtration with a 0.45 µm filter.

Sample preparation for CZE

The methods used for the extraction of histamine in the samples were based on Cinquina et al. (2004) and Numanoğlu et al. (2008). For extraction of canned tuna fish sample, 5 g of samples were transferred to centrifuge tube and 10 mL of 0.1

M HCl was added to the tube and homogenized using Turrax disperser for 2 min (Turrax T18 Basic IKA, Germany). After homogenizing, samples were put in an ultrasonic bath for 15 min (Bandelin Sonorex RK156, Germany), centrifuged at 10.000 rpm for 15 min (Jouan MR 1822, France) and filtered through a Whatman No. 1 filter. The supernatant fluid was separated from solid residue. This procedure was repeated twice for each residue. Both of the supernatants were transferred to a 20 mL flask. The flask was filled up to 20 mL with 0.1 M HCl, filtered and injected into the CZE-DAD System.

Results and Discussion

The level of histamine in canned tuna fish samples was determined by using ELISA. Afterwards, eight positive samples were reanalyzed by capillary zone electrophoresis technique. For both methods, all analyses were repeated twice for each sample. The results of the analyses were evaluated in accordance with the Turkish Food Codex values and European Commission Directives (EC, 2005; TFC, 2011). The histamine levels obtained in the samples were within the standard value (200-400 mg kg⁻¹) being the limit of Turkish and EU legislations. The mean pH values (\pm SE) of the samples for the A, B, C and D brands were determined to be 5.89 ± 0.02 , 5.86 ± 0.01 , 5.83 ± 0.02 and 5.82 ± 0.02 , respectively. The pH value in canned fish samples was found within value recommended by Turkish Standard Institute (4.0–6.9) (TSI, 2010). These values are shown in Tables 1 and 2. Analytical parameters of proposed CZE method for histamine detection are shown in Table 3.

For CZE method, the mean recovery was found 108 % in canned tuna fish. The limit of detection (LOD) and limit of quantitation (LOQ) were determined as 1.55 mg kg⁻¹ and 5.18 mg kg⁻¹, respectively. Percentage relative standard deviation (RSD%) of mean peak area was found 8.01 % for the interday (n=3) precision test and 1.9 % for the intraday (n=5) precision test. The calibration graph was linear in a range of 5-200 μ g mL⁻¹ with a regression equation, $y=0.25x+0.017$ ($r=0.9998$).

Table 1
Levels of histamine in samples of canned tuna fish as determined by ELISA

Brands	<2.5, mg kg ⁻¹	2.51-4.0, mg kg ⁻¹	4.1-10.0, mg kg ⁻¹	10.1-30.9, mg kg ⁻¹
A	16	1	1	2
B	16	2	-	2
C	20	-	-	-
D	20	-	-	-
Overall	72	3	1	4

The electropherogram of histamine-free and histamine containing (spiked with 50 ppm) sample is shown in Figure 1.

Table 2
Levels of histamine in positive fish samples as determined by ELISA and CZE

SAMPLE Code No (Brand)	ELISA, mg kg ⁻¹	CZE, mg kg ⁻¹
7 (B)	3.07	n.q
11 (A)	3.9	n.q
21 (B)	2.6	n.q
26 (A)	18.6	21
32 (B)	13.2	12.5
34 (A)	5.4	n.q
39 (A)	30.4	32.3
48 (B)	10.6	12.5

n.q: not quantitation

Table 3
Analytical parameters of proposed CZE method for histamine detection

Parameters	Value
Linearity (r)	$r=0.9998$
Regression equation	$y=0.25x+0.017$
Limit of Detection (LOD)	1.55 mg L ⁻¹
Limit of Quantitation (LOQ)	5.18 mg L ⁻¹
Repeatabilities	
Intraday repeatability (n=5, RSD%)	1.90%
Interday repeatability (n=3, RSD%)	8.01%

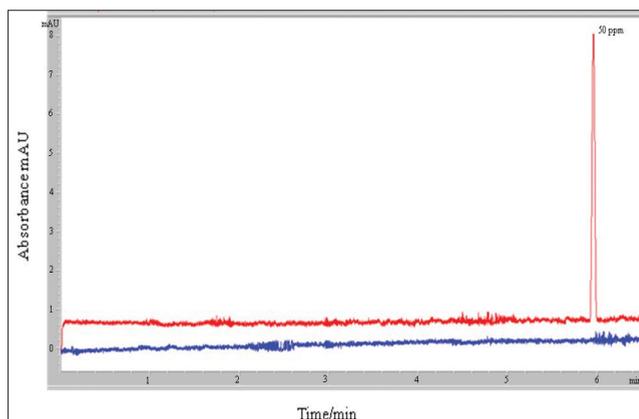


Fig. 1. Capillary zone electropherograms of the histamine-free and histamine containing (spiked with 50 ppm) sample under optimum pretreatment conditions and separation conditions (NaH₂PO₄·2H₂O and Na₂HPO₄·2H₂O separation buffer and 25 kV separation voltage)

The detection limit of ELISA was found to be lower than the CZE method. Thus, CZE method forms an alternative choice for the measurements of histamine in canned tuna fish. Muscarella et al. (2005) indicated that the ELISA test was simple and sensitive for histamine in seafood. The histamine levels in the canned fish samples were higher than the other previous surveys performed in Turkey. Varlık et al. (1992) estimated that the histamine levels of canned tuna fish were in the range 8.7-43.7 ppm. Similarly, in present study, the histamine levels were found to be 2.6-30.4 mg kg⁻¹ in canned tuna fish samples. Gökoglu and Varlık (1995) determined concentrations of histamine in first, second, third and fourth groups of canned sardine as 0.75, 3.46, 4.46, 1.87 ppm, respectively. Duyar and Ekici, (2011) reported that histamine content of twelve canned tuna fish were in the range 19.34-28.81 mg kg⁻¹.

The current study data is supported by previous studies on histamine levels (Kamkar et al., 2003; Kan et al., 2005; Auerswald et al., 2006; Smajlović et al., 2008; Rahimi et al., 2012).

Kamkar et al. (2003) reported mean levels of histamine as 10-178mg/100g in a total of 80 canned fish samples examined in Iran. Rahimi et al. (2012) reported histamine levels being as 17-210 mg/100g in Iran where 69.8% histamine rate in canned tuna fish samples were determined. Kan et al. (2005) indicate that no histamine could be detected in 63 canned tuna fish samples in Japan. Auerswald et al. (2006) have established that the levels of histamine in four canned tuna fish ranged between 0-2.4 ppm in South Africa. Smajlović et al. (2008) analyzed 23 samples of canned fish in Bosnia and Herzegovina and found less than 100 ppm of histamine levels.

Many foods naturally contain low concentrations of biogenic amines. The presence of biogenic amines at high levels may cause foodborne intoxications. Histamine is a very important chemical indicator in fish quality and sanitary controls. They are used as indices of bacterial fish spoilage. Sanitary and quality manufacturing are required to prevent histamine contamination in canned fish and thus the possible health hazards to the consumer.

Conclusion

In this study, 80 samples of canned tuna fish from different brands (A, B, C, D) were investigated for histamine. The mean levels (\pm S.D) of histamine were found to be 10.97 \pm 9.86 mg kg⁻¹ in canned tuna fish samples by ELISA procedure. Our data revealed that eight (10.0%) of canned tuna fish samples were positive for histamine. The histamine levels were within the Turkish Food Codex value and EU value. Howev-

er, the histamine levels of some samples were below the limit of quantitation in capillary zone electrophoresis technique. It is concluded that the low levels of histamine is explained by good quality of raw fish and hygienic conditions in canning technology. In conclusion present study shows that the histamine levels of canned tuna fish obtained during the period of study do not pose a health risk to consumers.

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